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THE BLACKPATCH DISEASE OF RED CLOVER AND OTHER LEGUMES IN WEST VIRGINIA[1]

J. G. Leach and Edward S. Elliott

(Accepted for publication July 20, 1951)

Blackpatch was first recognized in Kentucky and was reported in 1933 (2) as a previously undescribed disease causing large black leaf lesions on red and white clover. In wet weather it occurred in patches, but under less favorable conditions it appeared only on scattered individual plants.

Two years later it was observed in Wisconsin and in 1937 Smith (7) published a more complete description of the symptoms on leaves of red and white clover together with a description of the fungus and the pathological histology of infected leaves. On the basis of field observations and artificial inoculations, Smith concluded that the fungus was unable to attack any part of the plant other than the leaves. Infection was obtained by artificial inoculation on *Trifolium pratense* L., *T. repens* L., *T. repens giganteum* Lagr.-Foss., *T. incarnatum* L., *T. hybridum* L., *Melilotus alba* Desr., *M. officinalis* Lam., *M. indica* All., and *Medicago sativa* L. No infection was obtained on *Vicia faba* L., *Phaseolus vulgaris* L., or *Brassica oleracea* L. The pathogen was described as a sterile fungus with a coarse mycelium, hyaline when young but with age becoming dark green to brown and black. Since no spores or sclerotia were produced by the fungus, its taxonomic position could not be determined and it was not named.

In 1942 Chilton (3), reporting isolation of the fungus from two of 28,161 surface-disinfected seeds, suggested that, since the fungus was not known to produce spores or sclerotia, it might be transmitted from field to field on infected seeds.

The disease generally has been considered to be of minor economic importance and has received relatively little attention. In 1949 Elliott and Leach (5) reported heavy infection of blackpatch on red clover in eastern West Virginia, severely affecting the stems, flowers, and seeds and resulting in great reductions in seed yield. Weimer (9) reported the disease occurring in Georgia in 1950 on leaves of soybean (*Glycine max* (L.) Piper), cowpea (*Vigna sinensis* (Torner) Hassk., kudzu (*Pueraria thunbergiana* Benth.), and blue lupine (*Lupinus angustifolius* L.). Apparently no other work on the disease has been reported.

Blackpatch caused severe injury on red clover in certain sections of West Virginia in 1949. The purpose of this paper is to report certain significant facts about the disease, with particular reference to seed transmission of the fungus and the seedling blight that results from the planting of infected seeds.

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 436.

FIELD OBSERVATIONS

Smith (7) and Weimer (9) have pictured, on several hosts, the most conspicuous symptoms of the disease: dark brown or black necrotic leaf lesions. When the disease was first observed in West Virginia in 1948 on young, first-season red clover with few blossoms, leaf necrosis was the only symptom noted. In 1949 the fungus was observed attacking also stems, flowers, and seeds; there is good reason to believe, however, that these infections were present in 1948 but were overlooked, and that the disease was equally severe in that year. When the second-year crop was examined,

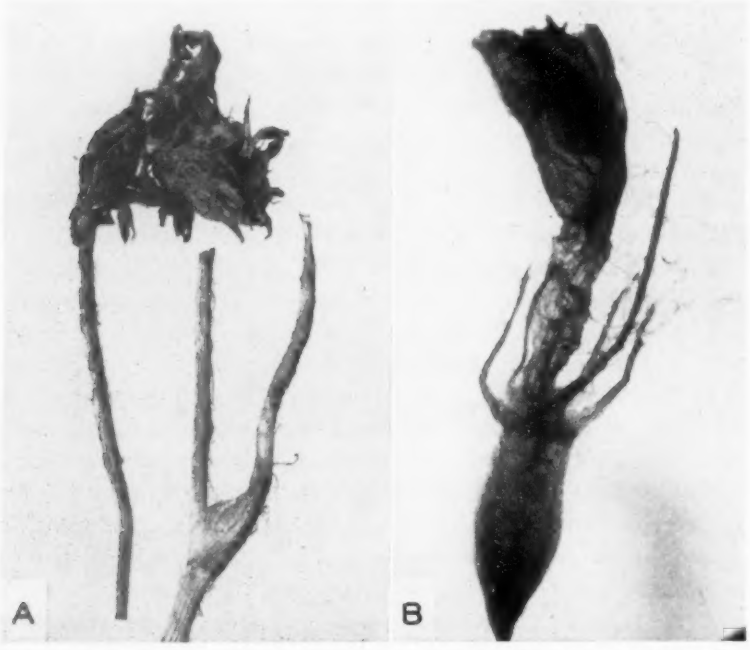

FIG. 1. Stems and flowers of red clover severely affected by blackpatch. A. A flower head and a section of stem showing the aerial mycelium that has completely overgrown them. Death of the flower head before maturity prevented the development of seeds. The stem has been girdled at several points. (Natural size.) B. A single flower from an infected head showing the mycelium that has destroyed it before the seed could mature. (Approx. 10 ×.)

flower heads were beginning to ripen and those affected with blackpatch were probably mistaken for heads turning black in the normal ripening process. Later aerial mycelium, easily confused with the normal heavy pubescence of many clover varieties unless examined with a hand lens, could be seen covering stems, blossom heads, and individual flowers (Fig. 1). Moreover, the clover was rather generally affected by blackstem (*Phoma trifolii* and *Cercospora* sp.), the stem lesions of which, unless examined critically, are readily mistaken for those caused by blackpatch. Farmers whose fields of red clover were heavily infected with blackpatch have been puzzled by what appeared to be sudden and rapid maturity of the plants.

It was obvious that the fungus, by girdling flower stems and by direct attack on immature flowers, was markedly reducing the seed yield of heavily infected fields. In order to obtain some measure of the amount of such reduction clover heads were collected at the time of seed maturity: 100 infected heads from a heavily infected field; 100 apparently healthy heads from the same field, and 100 heads from a field, apparently blackpatch-free, near by. These three lots of heads were threshed separately and the seeds of each lot divided into two parts, one containing all normal plump seeds and the other the diseased and shriveled ones that would normally be screened out in a commercial cleaner. The results are shown in figure 2.

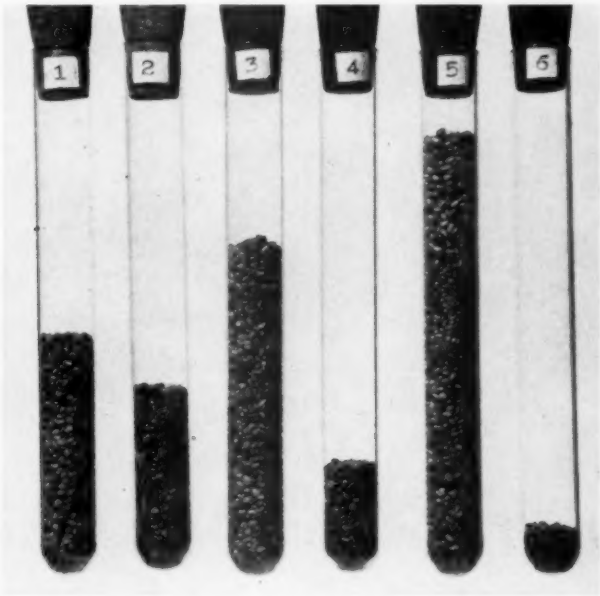

FIG. 2. Comparative seed yields of red clover with different degrees of blackpatch infection. 1. Healthy plump seeds from 100 infected heads selected at random from a field showing widespread infection. 2. Diseased and shriveled seeds from the same 100 heads. 3, 4. The corresponding yields from apparently healthy heads from the same field. 5, 6. Corresponding yields from 100 heads from a field in which no blackpatch could be found.

It is evident that the seed yield may be reduced at least 50 per cent as a result of blackpatch infection. The relatively high percentage of shriveled seeds from apparently healthy heads from a heavily infected field indicates that infection on other parts of the plant affected seed yield even though the heads were not directly infected.

SEED INFECTION AND SEED TRANSMISSION

In 1949 seeds from heavily infected clover heads, and seeds showing various degrees of infection by the fungus, were observed under a stereoscopic microscope.

Some seeds showed evidence of having been infected by the fungus while they were immature; they were reddish brown and badly shriveled. Fre-

quently traces of the coarse mycelium of the blackpatch fungus could be recognized immediately under the seed coat (Fig. 3, A). Other seeds appeared to have been attacked in various stages of maturity; some were shriveled and extensively discolored but most of them were of normal shape and color with moderate retention of chlorophyll green. Some were plump and apparently uninjured except that a coarse black fungus mycelium could be seen radiating out over the surface of the seed from a central invasion point at or near the hilum (Fig. 3, B).

Many of the badly shriveled seeds were nonviable but usually yielded the blackpatch fungus when plated on water agar. (Water agar has been

FIG. 3. Red clover seeds visibly infected by the blackpatch fungus. A. Ten seeds severely infected and badly shriveled. Coarse strands of black mycelium are visible beneath the seed coat. (Approx. 10×.) B. Nine seeds showing the black mycelium radiating over the surface of the seed coat. Such seeds are often plump or only slightly shriveled. Species of *Alternaria* and *Cladosporium* may also be found on the surface of such seeds but they are not pathogenic. (Approx. 10×.)

especially suitable for the isolation of this fungus; it grows readily on the agar, whereas many other fungi and bacteria make only sparse growth.) Some of these seeds showed unexpected vitality and germinated, but the seedlings usually were promptly killed by the blackpatch fungus.

Seeds not appreciably shriveled but with the blackpatch mycelium on the surface usually germinated normally but were soon killed by the fungus (Fig. 4, A).

Not all of the black mycelium visible on the surface of the seeds was the blackpatch fungus. Among others a species of *Alternaria* and one of *Cladosporium* were very common. In some cases the resting mycelia of these species were relatively coarse and black and, until cultures were made, diffi-

cult to distinguish from the mycelium of the blackpatch pathogen. These fungi, when present, made sparse growth on the old seed coat and in the surrounding agar and often grew over the roots and hypocotyl of the young seedling, but showed no indication of pathogenicity. The blackpatch fungus, however, would penetrate the seedling immediately on contact and kill it within a few hours.

When an infected seed germinated near a healthy seedling, both submerged and aerial mycelium quickly spread over the agar until the healthy seedling was reached and promptly infected; but in practically all cases

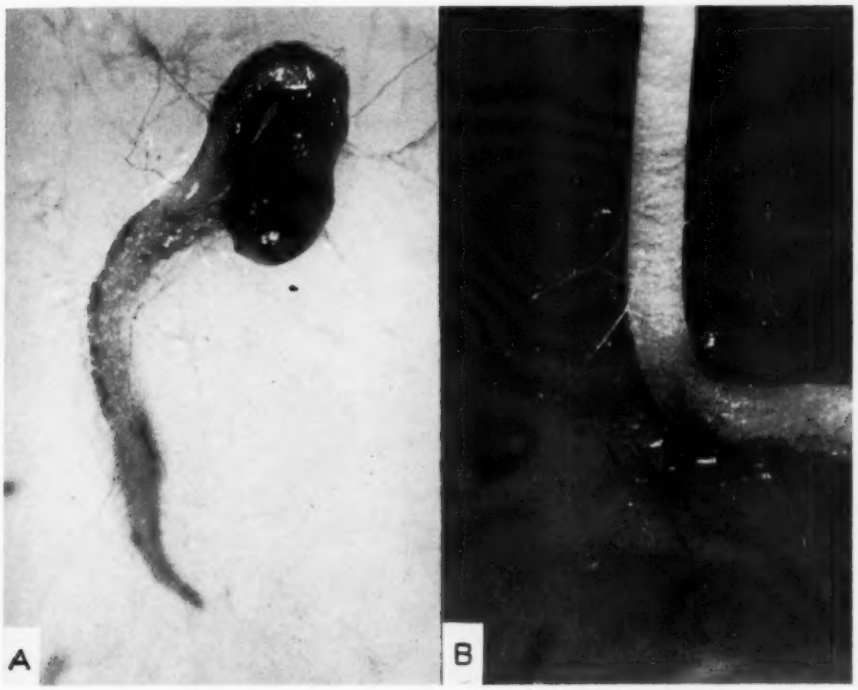

FIG. 4. A. A young seedling originating from a seed such as those shown in figure 3, B, after planting on water agar. The large mycelium of the pathogen which grew from the seed coat destroyed the seedling before germination was complete. (Approx. 10×.) B. A healthy seedling on water agar being infected by two strands of aerial mycelium of the blackpatch fungus.

the first infection was by long strands of dichotomously branched aerial mycelium which seemed to wave about until they came into contact with the seedling and penetrated promptly (Fig. 4, B). This process apparently is the principal means of spreading from plant to plant in the field, since there are no spores for disseminating the fungus.

When infected seeds are planted in soil the seedlings are promptly killed, and if the humidity is high the mycelium will, within a relatively short period, spread to adjacent seedlings and infect them. This seedling blight can be watched to advantage when the seeds are planted in white quartz sand and germinated in a moist chamber. Figure 5, A, shows a

seedling originating from an infected seed. The coarse black mycelium can be seen growing from the infected seed coat. Two branches of aerial mycelium are visible, one penetrating a cotyledon and the other infecting the hypocotyl. Another such seedling in a somewhat more advanced stage of infection is shown in figure 5, B.

Seeds selected under the stereoscopic microscope as being free of visible mycelium are usually not infected and produce healthy seedlings. The degree to which healthy and diseased seeds can be recognized by visual examination is shown by the results of germination tests of seeds selected in this

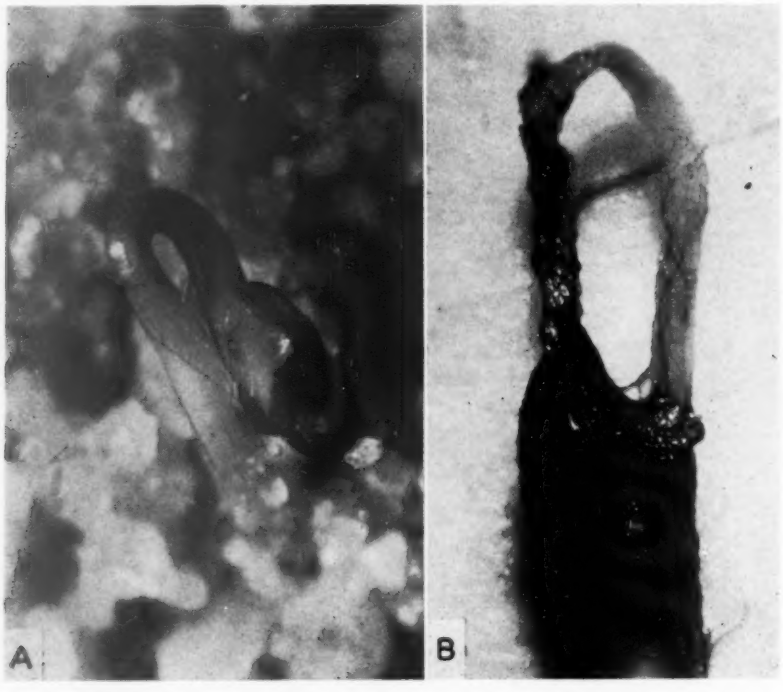

FIG. 5. Two red clover seedlings originating from infected seeds planted in quartz sand. A. Two strands of mycelium originating from the infected seed coat of a germinating seedling. One strand is infecting one of the cotyledons and the other the hypocotyl. (Approx. 10×.) B. A later stage of infection on a similar seedling. (Approx. 10×.)

way (Fig. 6). It was difficult to obtain accurate counts of seed infection from germination tests in soil or sand, for there were varying numbers of hard seed that did not germinate. Also, when seedlings were planted close together healthy seedlings often became infected by mycelium from adjacent seeds; but when healthy seeds and diseased seeds were adequately separated, the contrast was striking.

On the basis of seeds selected under the microscope as showing evidence of infection, and checked by germination on water agar, it is estimated that as much as 25 per cent of the seeds from an infected head, and from 1 to 3

per cent of a lot of field-run seeds from a heavily infected field, might be infected.

It is evident from these observations and experiments that blackpatch may, under favorable conditions, attack severely the stems, flowers, and seeds of red clover and greatly reduce the yield of both hay and seed. The fungus is seed-transmitted and may cause a blight not only of those seedlings arising from infected seeds, but also of seedlings from noninfected seeds that may be growing in close proximity to infected seeds.

PRELIMINARY SEED TREATMENT EXPERIMENTS

Since the blackpatch fungus is seed-transmitted and may cause a seedling blight, the possibility of control through seed treatment is indicated. Among reported experiments with red clover and other forage legumes (1, 4, 6, 8), some have shown increased stands resulting from certain seed treatments; others have given no significant differences. In no reported ex-

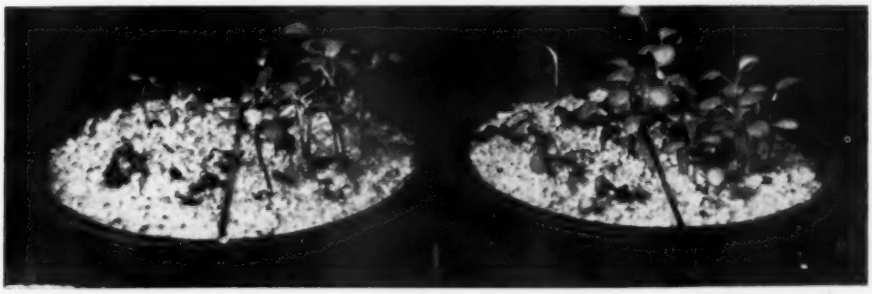

FIG. 6. Blight of red clover seedlings caused by blackpatch arising from infected seeds (left half of pots), compared with healthy seedlings from seeds selected by microscopic examination (right half of pots).

periment, however, has blackpatch been recognized as a factor in reducing stands in untreated seed lots: increased stand has been the criterion of the value of seed treatment. Where blackpatch is concerned a seed treatment, if successful, would have a theoretical value over and above that resulting from increased stands. Since the blackpatch fungus produces no spores and must spread from plant to plant by vegetative mycelium, its prevalence in a field would depend largely upon the number of infection centers arising from infected seeds. This would certainly be true when clover is planted on clean soil and would probably be true on all soils unless the fungus survives more extensively in soil than available information indicates.

At the present time results can be reported only for small-scale greenhouse experiments with seeds from a field heavily infected with blackpatch. Those were selected which showed visible injury when examined under the microscope, indicating probable infection by blackpatch. Platings made on water agar from such representative samples indicated that 20–30 per cent of the seed was infected. The selected seeds were divided into two lots: one lot was dusted with Arasan (1 part to 100 parts of seed weight); the other was not treated. The seeds were planted in white quartz sand in

3-in. pots and the pots were placed in a greenhouse moist chamber until the seeds had germinated and made considerable growth.

Three different plantings were made at different times, all with comparable results. The data on one typical experiment are in table 1.

In every case the number of seedlings emerging from the treated seeds was greater than the number emerging from the nontreated seeds. In this experiment consisting of eight lots of 40 treated seeds in comparison with eight lots of 40 nontreated seeds, the totals of emerged seedlings for each lot of 320 seeds were 191 and 110 respectively. This represents an increased stand of approximately 73 per cent resulting from seed treatment. But, since the treated seeds contained a higher percentage of infected seeds than would be found in most commercial seed lots, increased stands of this

TABLE 1.—*The effect of seed treatment with Arasan on the development of blackpatch on red clover seedlings*

Arasan treatment			No treatment		
Pot number	Seedlings[a] emerged	Blackpatch development	Pot number	Seedlings[a] emerged	Blackpatch development
2	21	None	1	12	Early: rapid spread
4	23	None	3	7	Early: rapid spread
6	14	Present: slow spread	5	13	Early: rapid spread
8	27	Late: slow spread	7	15	Early: rapid spread
10	24	None	9	10	Early: rapid spread
12	21	None	11	12	Early: slow spread
14	21	Late (1 plant)	13	15	Early: slow spread
16	21	None	15	16	Early: rapid spread

[a] Forty seeds planted: some were not viable; others were hard seeds and did not germinate.

magnitude would not be expected from seed treatment as a general farm practice. However, as previously mentioned, the value of seed treatment for blackpatch cannot be measured by increased stand alone. In fact, the greatest value is likely to be found not in the total stand but in the reduced number of infection centers consisting of infected seedlings from which the fungus may spread to adjacent plants and increase correspondingly the amount of infection on older seed-bearing plants. Since the fungus produces no spores and spreads in the field only by aerial mycelium, a reduction in the number of infection centers arising from infected seedlings would probably influence considerably the prevalence of the disease in a field in late summer. This probability is supported by the early development and rapid spread of blackpatch on the plants emerging from nontreated seeds as compared with its absence or late development on plants from treated seeds.

Further experiments on control of the disease by seed treatment and other methods are under way.

SUMMARY

Blackpatch of clover and other legumes, previously considered to be a disease of minor importance affecting leaves only, has been very destructive on red clover in certain sections of West Virginia. Under humid conditions the fungus is a virulent parasite on leaves, stems, flowers, and seeds. In heavily infected fields of red clover the seed yield may be reduced at least 50 per cent. The fungus is seed-transmitted and causes a severe seedling blight.

Since the fungus produces no spores it is dependent upon aerial mycelium for spread from plant to plant in the field. Thus seed transmission is of more than ordinary significance, because the prevalence of the disease in a field will be influenced greatly by the number of infection centers arising from infected seeds.

Preliminary small-scale experiments indicate that the number of infection centers and the amount of seedling blight may be greatly reduced by seed treatment with Arasan.

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LITERATURE CITED

1. ALLISON, J. LEWIS, and J. H. TORRIE. Effect of several seed protectants on germination and stands of various forage legumes. Phytopath. **34**: 799–804. 1944.
2. ANONYMOUS. Blackpatch of clover. Ky. Agr. Exp. Sta. Ann. Rpt. 46. 1933.
3. CHILTON, S. J. P. Some pathogenic fungi occurring in the seed of red and subterranean clover. Phytopath. **32**: 738–739. 1942.
4. ————, and R. J. GARBER. Effect of seed treatment on stands of some forage legumes. Jour. Amer. Soc. Agron. **33**: 75–83. 1941.
5. ELLIOTT, EDWARD S., and J. G. LEACH. "Blackpatch" as a limiting factor in red-clover seed production in West Virginia. (Abstr.) Phytopath. **40**: 7. 1950.
6. KREITLOW, K. W. Investigations on seed treatment of forage grasses and legumes for control of damping-off. U. S. Dept. Agr., Pl. Dis. Rptr. **27**: 111–112. 1943.
7. SMITH, OLIVER F. A leaf spot disease of red and white clover. Jour. Agr. Res. [U.S.] **54**: 591–599. 1937.
8. VLITOS, A. J., and D. A. PRESTON. Seed treatment of field legumes. Phytopath. **39**: 706–714. 1949.
9. WEIMER, J. L. Blackpatch of soybean and other forage legumes. (Abstr.) Phytopath. **40**: 782. 1950.

FIELD TESTING OF FUNGICIDES FOR VEGETABLE DISEASE CONTROL

J. D. WILSON

(Accepted for publication July 25, 1951)

In the testing of a new fungicide, the final step in its development is usually a field comparison with others in more common use with respect to its comparative ability to control disease and its degree of phytotoxicity. These comparisons are commonly made on more than one crop, for the control of more than one disease, during more than one season, and in several geographical locations. The minimum quantity of the active ingredient to be used in either spray or dust formulations for a satisfactory degree of disease control must be determined. Also, it is desirable to observe the degree of compatibility with any and all insecticides with which it may be used.

Sometimes a potentially good fungicide fails because of improper formulation. Thus, if the trial compound does not give the degree of disease control which was expected of it on the basis of preliminary tests, different basic formulations may have to be prepared and tested. The possible use of adjuvants to improve its performance should be investigated also.

If the new material promises to be more costly per control unit than other materials already on the market, then it becomes important to very carefully determine the minimum quantity which will consistently give good disease control and thus make it better able to compete pricewise. For instance, copper 8-quinolinolate, one of the most promising fungicides of recent introduction (4), had a minimum cost of manufacture so high that it could not compete on a cost basis with other less efficient but cheaper materials. With these points in mind the investigator may next turn his attention to the techniques of field procedure.

The many considerations involved in planning an experiment on the field testing of fungicides have been divided, for the purpose of discussion, into those pertaining to site, crop and variety, planting techniques, application equipment, formulation, application schedules, the taking of preliminary data followed by yield and supplemental data, the statistical treatment of these data, and finally the publication of the results and possibly recommendations for use.

SITE

Several factors must be considered in the selection of a site that will be suitable for an experiment dealing with the comparative testing of fungicides or fungicidal formulations on the basis of their ability to control disease.

Soil type and uniformity. A soil type inherently suited to the growth of the crop to be treated should be selected, avoiding, for instance, a heavy

clay loam for potatoes or a light sand of low fertility for cannery tomatoes. It also is important that unless the soil type is comparatively uniform over the whole area of the experiment, another variable is added to the already large number usually accompanying an experiment of this type involving a considerable number of small plots.

Drainage (soil and air). Good soil drainage, either natural or artificial, or both, is essential and must be evenly good over the whole area, removing any excess of water within a few hours. Potatoes, for instance, may be injured irreparably if any free water remains on the surface or in the first foot of soil for more than a few hours. The site should be so located that surface water from higher elevations does not run across or over the plots. The location of tile lines should be considered in laying out and arranging the experiment as a whole so that small individual plots are not unduly affected by the location of any portion of the drainage system.

Some drainage of air across the experimental area, with no pockets or depressions where cold air may be trapped, is important, especially with respect to a disease like late blight of potato and tomato which is very dependent on low temperatures and high air humidities for its initiation and development. High shrubs or a group of trees along one or more sides of the area may interfere with air drainage.

Fertility. The experimental area should be of at least average fertility with a good balance of nutritional elements but no marked excesses or deficiencies. If specific experiments require plants of other than normal growth characteristics, special provisions may be required.

Location. This is not necessarily of great importance, but the site should be sufficiently accessible so that spray or dust applications may be made at the proper time, and that unexpected breakdowns in equipment may be repaired without undue delay. Also, it frequently is desirable to select locations widely enough separated geographically to afford a comparison of fungicidal performance on plants grown under very different soil and climatic conditions.

Size and shape. The size of the area must be sufficient to include all the plots, of whatever size and shape, needed for not less than four and perhaps as many as six or ten replications of each of the treatments to be compared. The size and shape of the area as a whole may be further influenced by the type of plot arrangement that will best fit the situation with respect to the actual application of the different materials. This is especially important when a great many treatments must be made in one day —it seldom being desirable to continue a series into the second day because of probable changes in the weather. Under these circumstances it is important to keep to a minimum the distance traveled by the application equipment and the various supply units.

The size and shape of the individual plot replicates are frequently determined by circumstances, and usually represent a compromise between what the investigator would like to use, the available space and labor, and

perhaps the minimum size that can be used and still provide the necessary degree of assurance that the results will be mathematically valid. If at least four replicates are used, vegetable plots seldom need to be over 1/20 acre in extent and may be as small as 1/300 acre, depending on the type of application equipment being used. Their shape is frequently determined by the type of crop being grown and the tread spacing and boom length of the spray or dust equipment. Thus, the plots are usually long and narrow rather than square; as short as 25 ft. for celery, as long as 60 ft. for potatoes, and from 4 to 6 ft. wide, if tractor-mounted equipment is used.

CROP AND VARIETY

Type of crop. The crop on which a given fungicide or group of fungicides is to be applied must be considered. For instance, a study of the ability of a fungicide to control one or more of the vegetable diseases caused by the genus *Colletotrichum* would have to be made on a crop or crops subject to attack by that group of fungi, such as tomato, bean, muskmelon, etc. For an effective control material for late blight, the crop choice would probably be tomato or potato. Fungicides capable of checking *Alternaria* would be applied to various members of the Solanaceae susceptible to attack by this group, such as potato, tomato, pepper, eggplant, etc. Many examples of this sort of specificity exist.

Variety. The matter of crop selection is frequently extended to include a consideration of the variety best suited to a study of fungicide performance. For instance, some varieties of tomatoes are more susceptible to early blight and to anthracnose than others, some varieties of celery are more severely attacked by early blight than others, and similar examples might be given for such crops as potato, carrot, cucumber, etc. Thus after selecting a test crop which is subject to attack by a particular disease one should select a variety of that crop which is especially susceptible. Any fungicide that is selected as effective on special varieties will, of course, have to prove itself in general use on standard varieties.

PLANTING PLANS

It is necessary to consider planting distances between rows or plots, and between plants in the rows if transplants are used; length and breadth of individual plots; the number of replicates to be used for each treatment and their arrangement or location with respect to one another; and width and location of marking alleys and transverse roadways for the movement of application equipment.

A design or plan for the arrangement or location of the different replicates of each of the treatments to be used must be selected or developed for each experiment. Some type of random arrangement is usually desirable for any experiment which includes over 10 treatments. A Latin square or some modification of this may be used when not over 10 treatments are involved. If special considerations with respect to analysis of

the data are involved, a trained statistician should be consulted. Special designs are already available or can be devised to fit almost any situation (2, 3).

The author has found the randomized block one of the most adaptable to this type of experimentation. The design is comparatively flexible and although it is preferable that the test area be square, this is not strictly necessary (1) and frequently is not practical. It is usually desirable to arrange each block of replicates with roadways between so that application equipment may be moved transversely across the ends of the plots. This will allow the equipment to travel from the end of one treatment replicate to the beginning of the next, and so on, with the result that all replicates may be treated in one round trip. The identifying stakes should be so placed that they are always at the end from which each replicate of a given treatment should be approached. To further facilitate this plan, the supply equipment may be moved to the location of the first plot replicate of the next treatment to be applied. This plan reduces the application time sufficiently so that as many as 40 different treatments may be applied to six replicates in an 8-hr. day, even if the equipment moves at no more than 2 miles per hr. while applying the spray material.

A new type of plot stake now in use by the author might be worth a short description. The stakes may be stamped from 20-gauge aluminum or from galvanized iron. One type is about 18–20 in. long, pointed at the lower end and tapering gradually to a width of about 4 in. at a place about 5 in. from the top. The upper portion consists of a rectangular area about 5 in. high and 4 in. wide. Two tabs or ears on each side and at the bottom of this portion act as a holder for an aluminum target or plate about 3 by 4 in. The plot identifications are painted on these targets with waterproof ink, which can be removed at the end of each season, permitting the use of the target over and over. A U-shaped groove is stamped lengthwise in each stake to provide rigidity. They may be assembled in bundles for storing or handling in such a way that 25 or more may easily be held in the hands, and several hundred may be stored in a box 3 ft. square and 3 ft. deep.

EQUIPMENT

Too many points are involved for complete discussion here in the design and operation of equipment for application of experimental fungicidal formulations as sprays, dusts, or concentrates. However, it is highly desirable that the best equipment available be used.

Mechanically propelled sprayers or dusters, which are preferable since they remove much of the human element, require a speedometer to determine and regulate the rate of travel during the time the material is actually being applied to the experimental plot. A comparatively inexpensive speedometer is now available for farm tractors and an old automobile speedometer may be adapted for use on other types of self-propelled equipment.

Sprayers. Pump pressures and delivery rates should be adequate and

capable of easy and accurate regulation by means of a good relief valve and a pressure gauge. Booms should be so designed and constructed that the proper nozzle arrangement for maximum coverage on any type of crop may be obtained quickly and maintained easily. The nozzles should be carefully selected to do the job required of them, and a means of obtaining a positive stoppage of flow when the pump is shut off also is desirable. Plentiful agitation for the sprayer tank is especially important for experimental use, since many of the new formulations submitted for comparative testing are undependable as to wettability, suspensibility, etc.

Dusters. If a duster is to be used, several other problems must be considered, since accuracy in application is even more difficult to obtain with dusts than with liquids, though just as necessary. The weaknesses of the average commercial duster are legion, but the fundamental requirements of a dependable machine are good agitation to prevent packing of the dust mass in the hopper and to prevent bridging of the feed opening, an even rate of feed at all hopper levels, a means of obtaining and maintaining any selected rate, and finally the even distribution of air and an unfractionated dust mass to each outlet. Few if any dusters now on the market possess all of these characteristics, although there have been marked improvements in design during the past few years.

Since the physical characteristics of two dust formulations are seldom if ever identical, each will tend to flow, or feed, from the duster hopper at a different rate. These inherent differences in the flowability of dust mixtures (5) make it highly desirable that the rate of flow of each test mixture from the duster which is to be used to apply it be determined before field operations are begun. If the feed-slot openings required to give a selected rate of flow for each mixture are recorded and then used each time a field application is made, the accuracy of comparative tests will be greatly increased. If known variations in outlet delivery exist it is often feasible to pair those of highest and lowest delivery, as well as those of successively lesser variation, in such a way that approximately the same amount of dust will be applied to each plot row. Finally, the standard precaution should be observed of applying dusts only during quiet periods and when the leaves are moist.

FORMULATION

Wettable powders. Most of the fungicides submitted for testing already have been formulated by the manufacturer. The majority are prepared as wettable powders, which are usually insoluble in water. However, a few are now being introduced as liquids. One of the weakest links in the chain of circumstances involved in the production and testing of fungicides has to do with formulation. The preparations as they are received may contain too little or too much wetting agent. If too little, they are difficult to wet, and if too much they foam excessively in the tank. In either case a suitable and uniform suspension is difficult to maintain

and the active ingredient is not evenly pumped from the tank and sprayed on the plant. If too much wetting agent is present the resulting deposit is too easily washed off by rain.

The matter of particle size of the active ingredient was formerly the subject of considerable discussion, but most of the new materials that are now submitted for testing have been prepared with the size of the individual particles as small as it is practical to make them.

Dust mixtures. Completely formulated dust mixtures are seldom prepared for testing by the manufacturer of the fungicidal ingredient. More commonly only the active ingredient is furnished, with recommendations concerning its formulation in a dust mixture. The specifications usually deal with the percentage of the active agent which the mixture should contain, the type of diluent such as clay or talc, and the amount and kind of adhesive agent if any is to be used. In actual practice it is probably desirable that the investigator prepare the experimental mixtures, since this affords him an opportunity to formulate a series of fungicides all in the same manner. As suggested above, the duster for applying the various mixtures should then be calibrated for each of the formulations in order that all may be applied at the same rate per acre in the field.

Combination with insecticides. The vegetable grower frequently wishes to apply a fungicide and an insecticide in a single operation. This means that the two types of materials must be intimately mixed either in the sprayer tank or in the duster hopper. The mixtures may have been prepared previously to the time of application, as in many dust formulations, or they may be made just as the spray (tank mix) or dust mixture is being made ready for application. In either instance the two or more materials used in a single formulation must be chemically compatible.

Since such crops as potatoes, cucumbers, and several others are usually treated with these combination formulations, it frequently is desirable to cooperate with an entomologist in performance tests of both types of materials. If such cooperation is not possible or feasible, likely combinations should still be prepared, and tested as such, in order that any incompatibilities may be discovered early in the experimental procedure.

Concentration. The concentration of the active ingredient to be used in a spray or dust formulation must be decided upon before field applications are begun. Frequently the manufacturer has previously determined approximately what this should be to furnish a satisfactory degree of disease control. The amount to be added to each 100 gal. of spray material, or in preparation of 100 lb. of a dust mixture, is based necessarily upon the use of a predetermined quantity of spray or dust per acre. Field tests frequently include concentrations slightly above and below the one thought most likely to be satisfactory. Many of the fungicides commonly used on vegetables have been formulated in such a way that from 2 to 4 lb. of the material are used in each 100 gal. of spray material, or 6–14 lb. in each 100 lb. of dust mixture.

APPLICATION SCHEDULES

Date of first and last application. The time of the first fungicide application is sometimes regulated by some other consideration than the grower's desire to obtain the best possible degree of disease control, such as the most efficient and economical use of the material used. Some diseases, such as early blight of tomato, seldom attack the plant severely until a certain ratio between fruit load and leaf area has been established. The first spray application designed to control anthracnose fruit rot of tomato, for example, need not be made until the first fruits are present, by which time some early blight, but seldom a critical amount, may be present.

The last disease-control application should usually precede harvest by a week or two, depending on the crop, the disease to be controlled, and the fungicide to be used. An example of the danger of quitting too soon is that late-blight tuber rot may appear, at the time of digging, in a potato field that was sprayed all season except for a last application that might have prevented a delayed infection of nearly dead vines.

Interval between applications. Factors such as certain growth characteristics of the crop plant, the durability and adhesiveness of the fungicide, and the potential activity of the disease-causing organism determine the proper interval between applications. It is always advisable to cover new plant growth as often as it is practical to do so. For instance, young potato plants which may grow several inches each week should be sprayed every 6 to 10 days. Celery, which grows rapidly at the center during the latter half of its growth period, should be treated at least every 6 days to protect it from infection by Septoria leaf spot, a disease which may be very active under certain weather conditions. Some fungicides, more durable in their protective action than others, may be applied less frequently to give comparable control: Bordeaux mixture, for instance, may protect against late blight of potato over a longer period than some of the less durable organic fungicides.

PRELIMINARY DATA

Host injury. It is always necessary to watch carefully for any appearance of injury to the crop plant from the direct action of the fungicidal ingredient or as a secondary effect of some added material—a wetting agent, a diluent, or an insecticide. Host injury may appear as marginal burning of leaves, spot necrosis, loss of green color, bronzing of leaf surface, or stunting or even deformation of leaves and stems. Blossoms and growing points may also be injured.

Initial infection and the resulting reduction in leaf area. The whole experimental area, and particularly the check plots, should be watched carefully as the season progresses for the appearance of any of the one or more foliage diseases to which any given crop may be susceptible. Inspections should be made at least weekly and the location of the first infection points recorded as they appear on the differently treated plots. When the

untreated check plots are judged to have lost at least 25 per cent of their effective foliage area through disease attack, the corresponding foliage condition for all plots should be estimated and recorded as the percentage of defoliation which has occurred (2). The average loss for each treatment should give a fairly good picture of the ability of each fungicide or formulation to control infection by any given disease or combination of diseases. If more than one disease is present, it may be desirable to assign a value to the damage done by each. Just how these disease or defoliation estimates are made is largely at the discretion of the pathologist doing the work (2), but they should be sufficiently thorough to give a reliable check on the comparative action of the different materials under observation. These estimates should be repeated at approximately weekly intervals until most of the plots are defoliated, or until harvest, whichever comes first.

YIELD AND SUPPLEMENTAL DATA

Yield records at or during harvest. It is not always necessary to make a weight or volume determination or a numerical count of yield at a single harvest or successive ones, but such records usually afford the most reliable basis on which to judge the performance of a fungicide with respect to its disease-control efficiency and any injurious effects it may have on the crop plant. If no disease appears, data relative to fungicidal efficiency do not exist. However, if there is any hint of phytotoxicity, yield data may become important. Many fungicides are actually more or less phytotoxic, but any unfavorable effect on the host plant may be observed only in the absence of disease, since the beneficial effects of disease control frequently hide any mildly injurious action. For instance, the untreated check plots of such crops as cucumbers, muskmelons, and carrots sometimes produce larger yields than any of the treated plots.

Supplemental data. The collection at harvest time of data other than those directly pertaining to yield is frequently desirable. For instance, in harvesting tomatoes, it may be desirable to determine the percentage of the crop affected by anthracnose or late blight, as well as notes on grade, quality, and color. The same is true of data relative to the percentages of melons, cucumbers, or bean pods affected by anthracnose; of yellows in carrots; or of rotted fruits of pepper and eggplant. A double set of yield records may aid in judging the effect of various fungicides on disease control. For example, the determination of the gross and trimmed weights of celery often affords a better picture of the comparative ability of a number of different fungicides to control early or late blight on that crop than either set of data taken alone.

STATISTICAL TREATMENT

After the data on yield, defoliation, etc., have been collected it is usually desirable that they be subjected to statistical treatment of one kind or another (1, 3). The value most commonly determined for data of this sort

is that of "Least Significant Difference." It is usually calculated at odds of both 19:1 and 99:1, but the former is the more useful in this type of experimentation. Significance at odds of 99:1 would hardly be expected in a group of materials all of which have previously survived laboratory screening tests.

Extreme care must be exercised to avoid mistakes in the collection of data from an experiment in which from 10 to 40 different fungicides, applied to anywhere from 4 to 6 or 8 replicates in a randomized design, are being compared. This is especially true with a crop like tomatoes or cucumbers which have to be picked five to ten times during a season. However, if the data are correctly taken and assembled, they then may be subjected to various kinds of statistical treatment for the determination of other values.

PUBLICATION

This, by virtue of the fact that the results must be assembled before they can be evaluated, is the last step in the chain of events involved in the field testing of fungicides or fungicidal formulations. It is not by any means, however, the least important or the most easily accomplished portion of the program. The preparation of annual progress reports on continuing phases of such an experimental or testing program frequently requires days of tedious preparation before a word is written. The finished tabular data must then be carefully inspected, while at the same time attention is given to various field notes, before a concise discussion of the results can be made. Following the completion of such a report, which is seldom published in a permanent form, it is usually desirable to prepare a more formal paper dealing with any highlights with respect to the performance of specific materials. This sort of publication may include changes in the standard recommendations for the control of specific diseases, if these seem to be justified by the results obtained over a period of two or three years. Finally, if the tests are carried on for several years, a publication which summarizes the results for that period may be desirable.

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LITERATURE CITED

1. COCHRAN, W. G., and G. M. COX. Experimental designs. 454 pp. John Wiley and Sons, New York, New York. 1950.
2. HORSFALL, J. G., and J. W. HEUBERGER. Measuring magnitude of a defoliation disease of tomatoes. Phytopath. **32**: 226–231. 1942.
3. LEONARD, W. H., and A. G. CLARK. Field plot technique. 271 pp. Burgess Pub. Co., Minneapolis, Minn. 1939.
4. WILSON, J. D. Fungicides and the control of tomato late blight, early blight, and anthracnose. Ohio Agr. Exp. Sta. Res. Circ. 1. 1948.
5. ————, and FRANK IRONS. Specifications of some of the ingredients commonly used in fungicidal dust mixtures. Ohio Agr. Exp. Sta. Bimo. Bul. 27: 26–41. 1942.

THE RELATION OF PSEUDOMONAS LACHRYMANS TO CUCUMBER FRUITS AND SEEDS[1]

A. B. WILES AND J. C. WALKER

(Accepted for publication August 10, 1951)

Smith and Bryan (4) published the first accurate description of the causal organism (*Pseudomonas lachrymans* (E. F. Sm. & Bryan) Carsner) of angular leaf spot of cucumber in 1915. They isolated the bacterium from infected cucumber leaves, grew it on artificial media, and obtained typical disease symptoms when they inoculated healthy plants with it and placed them under moist conditions. They failed to produce a fruit rot by artificial inoculation and concluded that the fruit-rot phase described earlier by Burger (1, 2) in Florida was incited by secondary rot-producing organisms which gained entrance into the fruits through the small epidermal lesions produced on the fruits by the leaf-spot bacterium. Carsner (3) reported results which were essentially in agreement with those of Smith and Bryan. He likewise was unable to correlate the leaf-spot phase of the disease with a destructive fruit-rot phase. Weber (5) in 1929, however, showed that fruit rot was often a common and destructive feature of the disease in Florida.

The present paper is a report of a study directed primarily toward the relation of the organism to the fruit and seed. The relation of the pathogen to leaf and stem was also re-examined.

METHODS AND MATERIALS

The movement and distribution of the causal organism in the tissues of the cucumber plant and in the fruits were ascertained by microscopic examination of stained sections. This examination was paralleled in most cases by isolations from adjacent portions of the host. The material used in this study was collected in the greenhouse and in the field principally on Chicago Pickling and National Pickling varieties of cucumber. Isolations were made using the dilution-plate method and *Pseudomonas lachrymans* was recovered consistently from lesions of the cotyledonary and true leaves, from stem and petiole lesions, and from various portions of infected fruits.

The plant tissues selected for histological study were killed and fixed by placing them directly in formol-acetic-alcohol. All tissues were evacuated under light suction while in the fixative. The material was dehydrated following the tertiary-butyl-alcohol schedule and embedded in paraffin according to the usual procedure. Sections, cut 10–12 microns thick, were stained in safranin (1 per cent in 50 per cent alcohol) followed by fast green (0.5 per cent in absolute alcohol). This combination was found to be

[1] This investigation was supported by a grant from the Wisconsin Pickle Packers' Association.

satisfactory, and the differentiation between the bacteria and the host tissues was generally clear and distinct.

EXPERIMENTAL RESULTS

Relation of pathogen to leaves and stems. The lesions produced by *Pseudomonas lachrymans* on cucumber leaves are at first minute, water-soaked spots, somewhat darker green than normal tissue. Under favorable environmental conditions these spots enlarge rapidly, and within 4–6 days may reach a diameter of 6–8 mm. They are generally angular in outline and appear to be delimited by the larger leaf veins. During periods of high humidity there often develops on the surface of the lesions a shiny drop of bacterial exudate. Under less humid conditions this exudate dries down to a white, chalky residue. As the lesions reach their maximum size the affected tissues begin to turn brown and to tear away at the margins from the surrounding healthy tissue. Shot-holing of the diseased leaves is produced by the falling out of these areas. The number of lesions per leaf is extremely variable and ranges from only a few to 50 or more. In cases of severe infection the spots often coalesce to form large areas of diseased tissue. At times the pathogen may invade the midrib or other leaf veins and cause puckering or downward curling of such leaves. Young leaves generally show a much greater susceptibility to the disease than do mature ones.

The pathological histology of diseased leaves was briefly discussed by Smith and Bryan (4). In the present study it was found that the pathogen invades the leaves and other host parts primarily through the stomata. Wounds may serve as an additional means of entry. Since stomata are most numerous on the lower surface of the leaf, the greatest amount of infection takes place in that area. In prepared slides of infected leaf material bacteria were observed in the openings of the stomata, in the substomatal chamber, and between the cells of the parenchyma. The bacteria were found in large numbers in the diseased areas of such leaves, but they were not seen in the adjacent healthy tissues surrounding the affected tissues. Lesion development was limited by the larger leaf veins. No invasion of the vascular tissues of the infected leaves was observed.

The petioles and stems of cucumber may also be invaded by *Pseudomonas lachrymans*. This type of infection often follows severe leaf infections, and occurs primarily under environmental conditions which are optimum for development of the disease. The organism gains entrance into these host parts through the stomata and occupies the intercellular spaces of the tissues beneath the substomatal chambers. Plasmolysis and subsequent breakdown of the parenchyma cells of this tissue follow. No bacteria were observed in association with the vascular tissues of infected stems and petioles, and none were found in apparently healthy petioles and stems bearing heavily diseased leaves.

Numerous attempts were made in the greenhouse to produce systemic

infection in healthy young plants by inoculating in the main stems through needle punctures. Usually no infection was obtained by this method and in a few cases small local cankers developed.

Relation of the pathogen to fruit and seed. When the pistillate blossoms and very young fruits are attacked by the pathogen they are generally shed from the vine in a short time. Fruit infection in the field is most often ob-

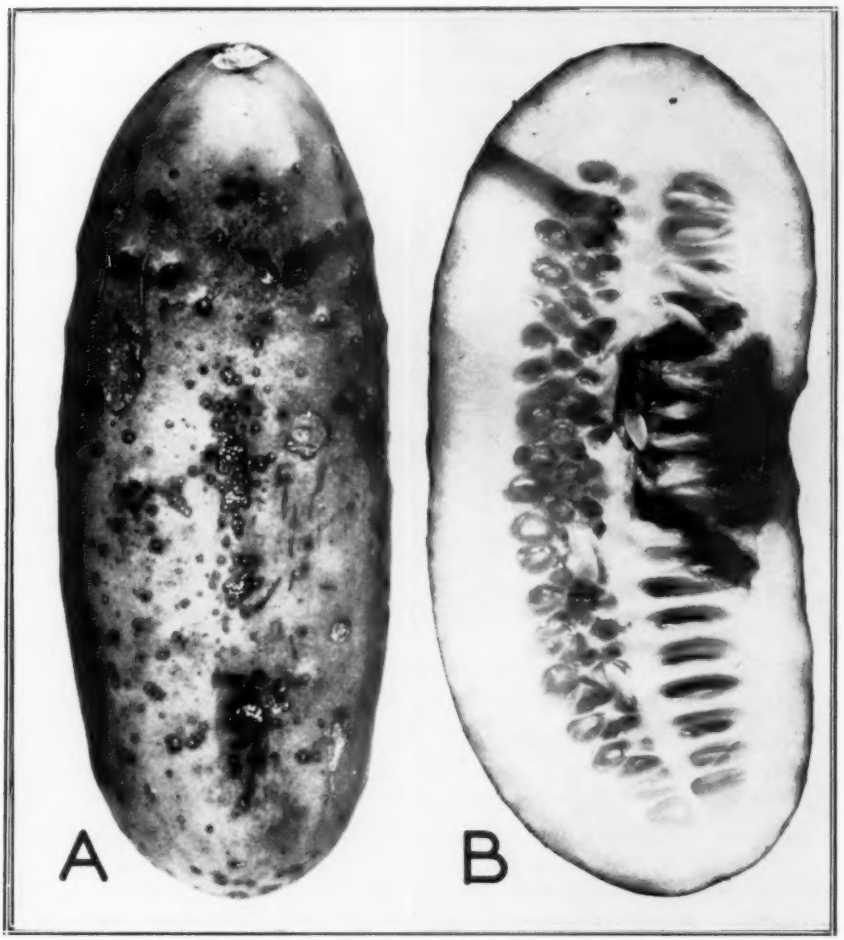

FIG. 1. A, small water-soaked lesions following stomatal invasion of a cucumber fruit by *Pseudomonas lachrymans*. B, longitudinal section of infected fruit showing restricted invasion of the hypodermal area and extensive breakdown of the middle and inner mesocarp.

served about the time that the fruits have reached half their normal size. The lesions are most numerous on the upper concavity of the fruit; they are small (2–3 mm. in diameter), circular, slightly sunken, and initially watersoaked (Fig. 1, A). Once formed, they do not increase appreciably in diameter, but there may be a coalescence of individual lesions. With

age the central areas become white or light tan and the epicarp turns back, giving the margin of the lesion a curled or fringed appearance.

Fruit lesions may vary from a few to several hundred per fruit and may be shallow or may extend into the affected fruit. Within the fruit a brownish discoloration of the tissues occurs (Fig. 1, B). In advanced stages this discoloration and breakdown extends the entire length of the fruit.

The organism invades the fruit through the stomata and initially occupies the stomatal openings and the substomatal chambers. The cells of the epicarp do not collapse immediately after invasion but remain intact as a thin membrane over the cavity. Eventually this tissue dries out and splits. In the early stages of invasion the bacteria are intercellular in the hypodermal region and a limited breakdown of the cells of this area follows.

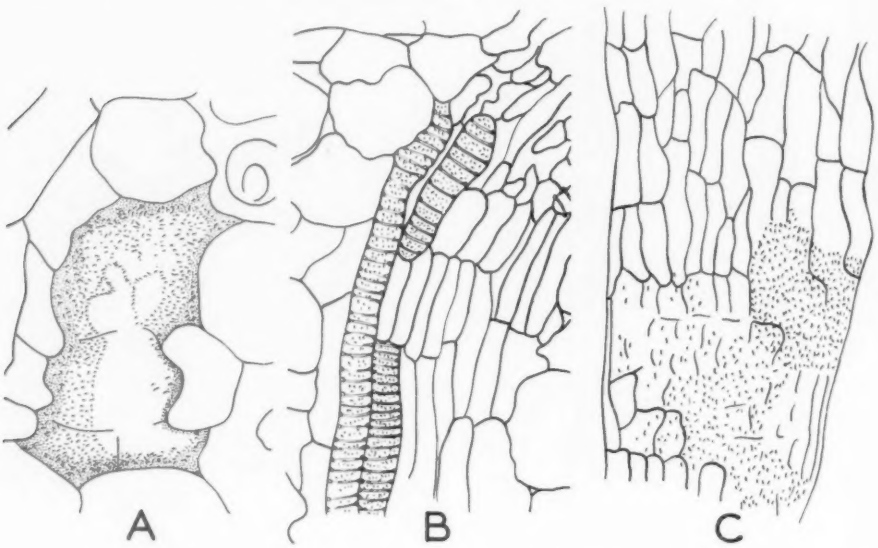

FIG. 2. Relation of *Pseudomonas lachrymans* to cucumber fruit tissue; drawings from photomicrographs. A, bacteria in and between thin-walled parenchyma cells of the middle mesocarp. B, bacteria within xylem elements of a vascular bundle in the mesocarp. C, bacteria advancing in and between the parenchyma cells of the funiculus.

In general, the path of invasion by the pathogen after entering the stomata is through the hypoderm and into the mesocarp with relatively little lateral invasion of the hypoderm. Once within the middle mesocarp region, which is composed of thin-walled cells with large intercellular spaces and numerous vascular strands, the bacteria advance throughout the entire length of the fruit. The organism occupied the intercellular spaces of this region (Fig. 2, A) and at times was found within the large xylem vessels of the vascular strands (Fig 2, B). It was also found intracellularly within ruptured parenchyma cells of the mesocarp. The fruit-rot phase of the disease is the result of the action of the bacteria in this region of the fruit. Attempts to demonstrate the presence of the causal organism within seed by histo-

logical methods were unsuccessful. However, the bacteria were shown by these methods to be present in the placental tissue of infected fruits and within the funiculus leading to the seed (Fig. 2, C).

Relation of the pathogen to the cotyledons. On the cotyledonary leaves of plants growing from naturally contaminated or infected seeds (Fig. 3, A) the lesions produced by the pathogen are variable in size and shape. They are often visible on the outer surface of the cotyledons as soon as it emerges from the seed coat. They may appear along the margins near the growing

FIG. 3. A, lesions on cotyledons of infected seeds in an early stage of germination. B, lesions on cotyledons at a later stage. Note the tissue in the center of some of the lesions has dropped out. Healthy plant on right.

point and on the inner surfaces of the cotyledon. These lesions develop in a manner similar to those on true leaves and likewise show a bacterial exudate during humid periods. The diseased areas eventually turn light tan or brown (Fig. 3, B). In the early stages of infection of the cotyledonary leaves the bacteria are entirely intercellular. Sections prepared from material fixed at this time show the stomatal openings and the substomatal chambers filled with the bacteria. In a short time the pathogen invades the intercellular spaces and begins to push apart the cells of the parenchyma shortly after the separation of the individual cells, the contents of the cells

become plasmolyzed, and the walls collapse or rupture. The bacteria may enter the cell lumen through such a rupture.

DISCUSSION AND SUMMARY

Knowledge of the relation of the angular leaf-spot organism to the fruit and seed of cucumber is essential for an understanding of the epidemiology of the disease. It is evident from the present study that progressive invasion of the mature fruits following earlier penetration of stomata is common. The bacteria advance intercellularly and within xylem elements of the mesocarp and in this manner eventually gain access to the seed. While the bacteria were not detected by histological means within the seed, they were demonstrated readily in the placental tissue and within the funiculus. There was circumstantial evidence from a study of germinating seeds taken from infected fruits that internal invasion of seeds occurs, since lesions appeared commonly on the surfaces of cotyledons emerging from the seed coat. Invasion of cotyledons, leaves, petioles, and stems appears to be restricted locally and no evidence of vascular invasion of vegetative tissues was secured.

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LITERATURE CITED

1. BURGER, O. F. A bacterial rot of cucumbers. Phytopath. **3**: 169–170. 1913.
2. ———. Cucumber rot. Fla. Agr. Exp. Sta. Bul. 121. 1914.
3. CARSNER, EUBANKS. Angular-leafspot of cucumber: dissemination, overwintering, and control. Jour. Agr. Res. [U.S.] **15**: 201–220. 1918.
4. SMITH, ERWIN F., and MARY KATHERINE BRYAN. Angular leaf-spot of cucumbers. Jour. Agr. Res. [U.S.] **5**: 465–476. 1915.
5. WEBER, GEORGE F. Angular leaf spot and fruit rot of cucumbers caused by Bacterium lachrymans E. F. S. & Bry. Fla. Agr. Exp. Sta. Bul. 207. 1929.

DEVELOPMENT AND PARASITISM OF PHYSODERMA GRAMINIS (BÜS.) FISCHER, ON AGROPYRON REPENS (L.) BEAUV.

K. S. Gopalkrishnan[1]

(Accepted for publication August 10, 1951)

A species of *Physoderma* first reported on *Agropyron repens*, from Madison, Wisconsin, by Thirumalachar and Dickson (18) in 1947 was believed by them to be morphologically identical with *P. graminis*, although they did not find resting sporangia in the root cortex cells. This species is reported from Germany, Denmark, France, Holland, and England on various grasses.

Physoderma graminis was reported by Childers (1) from Ottawa, Canada, in early 1949. Later in the same year the present author collected it in at least three new localities, in Michigan and Indiana, on *A. repens*. Absence of infection on other naturally associated grasses suggested that the fungus may be a distinct species of *Physoderma* or a physiologic race of *P. graminis*.

Since the closely related *P. zeae-maydis* causes considerable damage as brown spot of corn in the Southern States, further information on the life cycle and parasitism of *P. graminis* was needed.

MATERIALS AND METHODS

Diseased specimens of *A. repens* from Madison, Wisconsin, planted in the greenhouse, grew poorly but provided material for overwintering studies of the resting sporangia. *P. graminis* from Canada and *P. zeae-maydis* from Florida were propagated for comparison.

Germination was studied by Thirumalachar's method (17) in van Tieghem cells and by Ojerholm's method (12) slightly modified, the latter being particularly useful in reducing contamination. During summer even a suspension of sporangia in Syracuse dishes germinated satisfactorily.

Stains used were: cotton blue in lactophenol for temporary mounts, especially to observe the nature of the endosporangium during germination and the shape of the zoospores; Gentian violet, by Newton's method, for permanent mounts; neutral red and Janus green, intravitem, for comparison with fixed preparations and for differentiating special cellular structures. Host-parasite relationship, sporangial details, mode of rhizomycelial formation, and penetration were studied by using chrom-acetic-osmic-acid mixture fixatives followed by a triple stain and the iron alum hematoxylin combinations with Orange G as a counterstain. Feulgen reaction was successfully used in determining the chromatin nature of certain granular structures in the developing sporangium.

[1] The author is indebted to Dr. John A. Jump, under whose direction the study was undertaken, for valuable stimulating advice and constant encouragement. The work is part of a thesis submitted to the University of Notre Dame, for the Doctor of Philosophy degree. The author is grateful to the Tata Endowment, Bombay, India, for financial assistance.

Safranin fast green combination and Lepik's differential staining (9) gave satisfactory preparations for studying pathological anatomy and parasitism.

Trials in pots or Petri dishes determined the most suitable method and time of inoculation, preliminary to each of the final experiments in pots and small plots.

Seeds of *A. repens* were sown in the spring of 1949 in soil inoculated by two methods: 1) 1 oz. of finely powdered diseased leaves was mixed with 1 lb. of soil; 2) overwintered soil in which diseased plants had grown were mixed, 1:1, with sterile soil. Both soil and leaf powder were subjected to temperatures simulating natural winter conditions. Early stages of infection were observed on seedlings and peeled epidermis of the host inoculated by sporangial and zoospore suspensions. Of the various inoculation methods tried, spraying the host plants with zoospore and sporangial suspensions and incubating them for 72 hr. at 100 per cent humidity gave best results. Attempts to inoculate with a hypodermic needle were generally unsuccessful.

HOST SYMPTOMS

Shortened internodes and telescoped nodes of diseased plants change the usual spreading habit to an erect one. Yellow striae on the expanding pale young leaves gradually coalesce and become rusty brown. Leaves have a pink hue during the intermediate stages of the disease. Symptoms are restricted to the prominent leaf veins in moderately diseased plants, but are not so restricted in severe and late stages of infection. Leaves infected in the embryonic stage hypertrophy and thicken unequally, so that they are curled, twisted, or tubular when opposite margins of the leaf fuse. Chloroplasts in the leaf cells are often entirely replaced by sporangia of the parasite. Leaves infected later usually are larger than healthy ones (9.15 in. as compared to 6.25 in.). The erect habit and leaf yellowing make patches of infected quack grass readily recognizable in a well-mown lawn. Basal rotting of culms induces considerable wilting of affected plants. In contrast to all other species of *Physoderma*, this one forms no definite sori on leaves of *A. repens*. In the field, the fungus spreads centrifugally from an infection center each season, and the plants first infected gradually die.

Complete inhibition of the inflorescence suggests systemic infection, and this was confirmed by anatomical studies.

Symptom differences are not appreciable between samples from different localities. Probably a higher disease intensity was noted in the material at Notre Dame because of close and constant examination. Although *A. repens* has been reported from all the States, both the recorded southern limit of *P. graminis* and the northern limit of *P. zeae-maydis* are in Indiana.

LIFE CYCLE OF THE PATHOGEN

Efforts to cultivate the pathogen in culture media were partially successful when thiamine hydrochloride and yeast extract were added to the basic

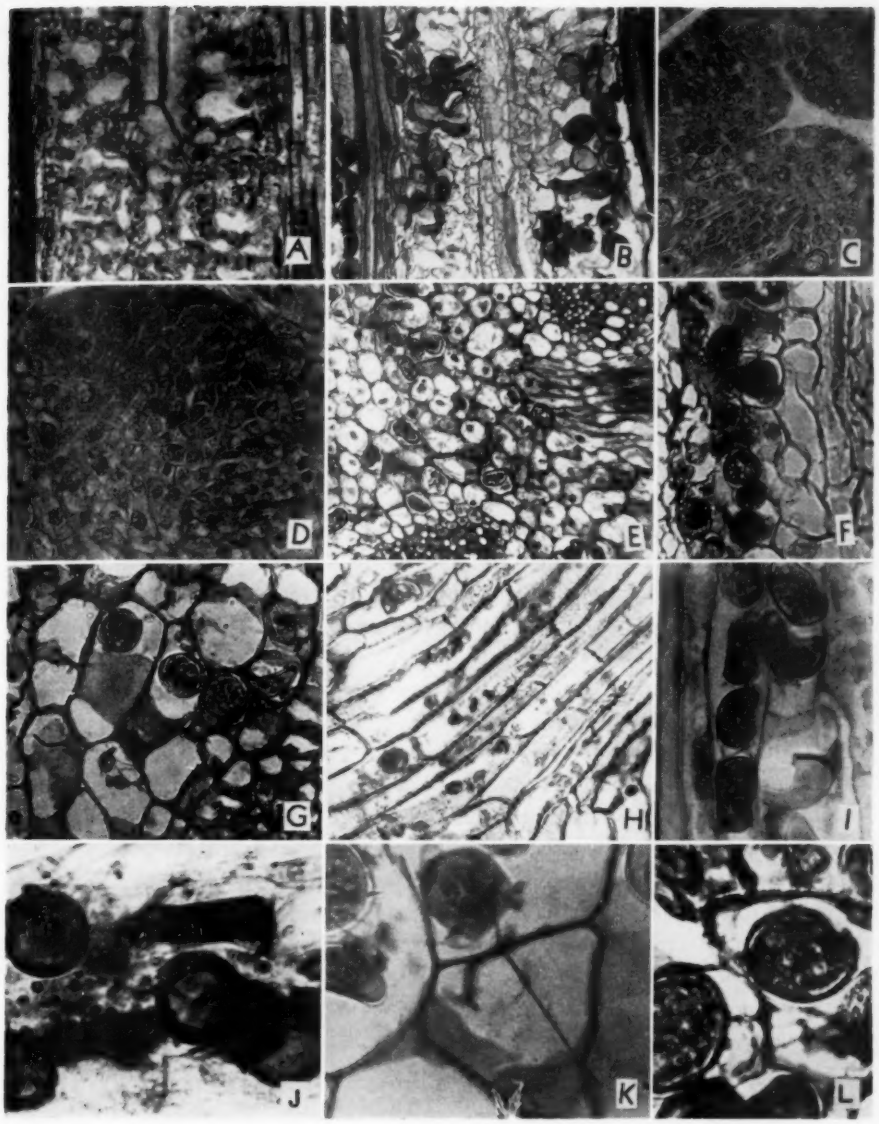

FIG. 1: A, B. Longitudinal section of leaf of *A. repens* (×300): healthy (A) and diseased (B); note the large number of sporangia in the vicinity of the principal veins. C. Infection of an embryonic leaf (× 300). D. Infection of the meristematic region (× 300). E. Resting sporangia in the rhizome (× 300). F. Endobiotic zoosporangia in section of the stem (× 300). G. Resting sporangia in section of the root; note rhizomycelium and two young Sammelzellen (× 600). H. Infection of the phloem and cells of the bundle sheath (×600). I. Section showing the chromatin concentration and distribution, in the developing resting sporangia (× 600). J. Group of resting sporangia releasing zoospores on epidermis of *A. repens;* note pairing of zoospores (× 1200). K. Penetration of the rhizomycelium through a cellulose wall (× 1200). L. Section showing the swelling of the cellulose walls where the rhizomycelia penetrate (×1600).

formulae of potato-dextrose and cornmeal agars. Bacterial contamination of the sporangial suspension was minimized with the addition of penicillin. The antibiotic had no effect on germination.

Infected parts of the host are brownish; orange-brown sporangia fill the cells, and the developing polycentric thallus completely destroys the chlorophyll. The Sammelzellen, one or more in a host cell (Fig. 1, I), may be transformed directly into sporangia, or may produce special branches bearing terminal sporangia. These short branches, resembling the rhizomycelium, may penetrate the cell wall and produce resting sporangia in a neighboring cell; these may be oval, round, or irregular, measuring 20–34 × 20–40 mu. A slight flattening of the wall on one side represents the region of germination of the sporangium.

The orange color of the sporangium is due both to the colored wall and to the contents. Light seems to have little influence; sporangia are brown even in subterranean portions of the host. There is an orange-brown exosporangium and a hyaline endosporangium; treatment with chlor-zinc-iodide colors the inner portion of the exosporangium and the endosporangium, but alcoholic phloroglucinol colors only the exosporangium. Thus it seems that the latter consists of an outer lignin and an inner cellulose layer, whereas the endosporangium is composed entirely of cellulose. The wall of the sporangium is 1–1.5 mu thick.

Longitudinal, paradermal, and transverse sections through the infected tissue were examined to trace the delicate rhizomycelium through the host cells. The sporangia, one to six per cell, have their contents arranged around a central globule appearing in the sections as an empty space. When sporangia are formed intercalarily from rhizomycelia they begin as ordinary enlargements, indistinguishable from the Sammelzellen. The available nutrition seems to be a deciding factor for an intercalary enlargement developing into a resting sporangium or a Sammelzelle: abundant nutrition may cause the enlargement to remain as a Sammelzelle giving rise to branches, and lack of nutrition may cause the investment of the enlargement by a dark wall and result in a resting sporangium.

Fresh sporangia under high humidity and at 28°–30° C. readily germinate in 36–72 hr. Germination is initiated by vigorous movement of the contents and gradual fragmentation of the central globule. At the end of 24 hr. there is circumscissile dehiscence and the lid is gradually lifted by the protruding endosporangium. At the end of 72 hr. the oval or watch-glass-shaped lid, which is of the same composition as the exosporangium and 10–12 mu in diameter, is finally pushed to one side or completely discarded.

The hyaline endosporangium may have one or two papillae through which the zoospores escape to the surrounding medium, or often the endosporangium itself may escape and then release the zoospores.

The spindle-shaped zoospores measure 2.5–6.5 × 2–4 mu, with a trailing posterior flagellum 15–25 mu long. An asymmetrical globule situated between the nucleus and the origin of the flagellum probably is of a fatty

nature because it turns black with osmic-acid treatment. Multiciliate zoospores occur as in other species (12). From 70 to 100 zoospores may be progressively released from a single sporangium. Motility is retained up to 24 hr. if the drop is free from contamination; otherwise the flagellum is lost within a few hours.

When such zoospore suspensions are placed on epidermis peeled from a leaf of *A. repens*, within a few hours the zoospores come to rest, produce irregular protrusions as illustrated by Tisdale (19), and give rise to rhizomycelial extensions penetrating the epidermal cells. Such penetration is often accompanied by production of rhizoids at the base of the attached zoospores, which are thus transformed into monocentric ephemeral zoosporangia. Such zoosporangia have been illustrated by Clinton (2) and Sparrow (13, 15) for other species of *Physoderma*. The zoospores released from these sporangia reinfect the host, thus enormously increasing the infection potential of the pathogen. These zoospores do not differ in size from those of the resting sporangia, and failure to observe fusions makes it difficult to assign a definite sexual function for the organ, although Sparrow thinks that it is a gametangium.

GERMINATION OF THE RESTING SPORANGIUM

In experiments to determine the age suitable for sporangial germination, using seedlings 7–10 days old, sporangia were fully developed 3 weeks from the time of infection, the first signs of the disease appearing after 2 weeks.

TABLE 1.—*Influence of light on sporangial germination*

Light	Percentage germination
Dark incubator	27.0
Moist chamber in diffuse light on laboratory table	54.0
Sunlight through a closed chamber in greenhouse	12.5
Sunlight in an open moist chamber in greenhouse	6.0
Moist chamber in a darkroom illuminated by a 60-watt bulb	43.0

In a series of experiments to determine viability of resting sporangia, those from material kept moist either with or without pretreatment at low temperature germinated over a long time, but pretreatment with low temperature hastened the attainment of peak in germination by 16 weeks. Drying the material reduced the germination significantly. Temperature suitable for germination ranged between 23° and 31° C. While sporangia could withstand dry heat up to 75° C. for a time, they were rendered inviable by moist heat of 35° C. Exposure of the sporangia to freezing conditions for 4 weeks or more did not affect germination adversely. Direct sunlight and darkness reduced germination (Table 1), and a free exchange of air in germination dishes was not essential for good germination.

Saturated salt solutions were used to control humidity in the germination chambers. At 98 per cent relative humidity (obtained with lead nitrate solution), 53 per cent of the sporangia germinated; and at 97.5 per cent humidity (with H_2SO_4 of 1.05 density), 38 per cent of the sporangia

germinated. None germinated when humidity was reduced to 93 per cent by a solution of disodium hydrogen phosphate. In the water controls, at 99–100 per cent humidity, 76.5 per cent of the sporangia germinated.

In dormancy studies, fresh sporangia collected in the autumn normally required 6 months before they could germinate. One series was exposed to $-6°$ C. for 6 weeks, another to alternate weekly exposures of $-5°$ C. and $+31°$ C., and a third set was left as control without temperature changes or treatment. Within 2 weeks after treatment at low temperature and treatment at alternating temperatures, 73 and 78 per cent of the sporangia germinated, whereas the control germinated only after 18 weeks and even then with a lower percentage. Amino acids like dl-histidine, isoleucine, and proline, growth regulators like phenyl acetic acid and 2,4-D, and 5 per cent sucrose and glucose solutions did not break the dormancy of the sporangia.

PATHOLOGICAL ANATOMY

The germ tube of the zoospore ends in a fine point which penetrates the epidermal cell wall. If the Sammelzelle is formed in the epidermal cell there is consequent enlargement of the cell. From this point rhizomycelia radiate to extend the parasite rapidly throughout the host tissue. The nuclear behavior is similar to that reported by Karling (5) for *Cladochytrium replicatum*, and sporangia germinated *in situ* (Fig. 1, J). The large surface of the folded Sammelzelle indicates a nutritive function.

When infection occurs early in the ontogeny of the leaf, the leaf remains folded, but if it occurs in later stages of leaf differentiation, infection starts at the base of the leaf and extends acropetally along the principal veins. Hence longitudinal sections show that cells near the vascular bundle, including the bundle sheath, contain the largest number of sporangia (Fig. 1, A, B). While phloem infection was common, no sporangial formation was observed, probably because of the abundance of nutrition (Fig. 1, H). This infection of the vascular system explains wilting, leaf yellowing, general stunting, nonflowering, and rapid spread of the disease in the host. Infection induces various degrees of cell enlargement, without stimulating cell division. The enlargement of cells is very pronounced in infections of early stages of foliar differentiation. The Sammelzellen have two to three cells. The single parent nucleus undergoes divisions and some of the daughter nuclei migrate into the extending rhizomycelia to organize new Sammelzellen or sporangia. With the formation of these new centers the parent Sammelzellen shrivel, possibly from lack of nutrition in the host cell.

Paradermal sections were studied to follow the course of the highly twisted and devious rhizomycelium. Wherever it penetrated a cellulose wall, swellings approximately twice the average diameter of the rhizomycelium and comparable to rust appressoria were observed on either side (Fig. 1, K). In a few cases the cell wall also showed hypertrophy at the point of entrance of the rhizomycelium (Fig. 1, L), suggesting an enzymatic action.

While it was difficult to find the rhizomycelium in the meristematic regions because of density of cell contents, fully formed resting sporangia were found about three to four cells below the actively dividing region (Fig. 1, D). These findings substantiate the systemic nature of parasitism already suggested by the symptoms.

Cross sections through the roots show abundant infected cells with sporangia in various stages of development (Fig. 1, G). Thirumalachar and Dickson did not find root infection. Therefore they were unable to verify the identity of the fungus, although on the basis of sporangial morphology they rightly suggested that it was probably *P. graminis*. The present study establishes that the pathogen in question is *P. graminis*, probably a variety different from that reported from Europe on *A. repens* and other grasses (10). There was no appreciable enlargement of the infected cortical cells of the root and no infection was found in the stele. Thin-walled oval or round bodies with densely staining contents were often associated with the resting sporangia in cortical cells (Fig. 1, F). No definite rhizomycelial connections were found between these and the resting sporangia of *P. graminis*.

Polymyxa graminis, a plasmodiophoraceous parasite, has been recorded in the roots of *Triticum durum* and other cereals by Ledingham (8). Sparrow has found it associated with *P. claytoniana*. The structures found in the present study differ from those described by these authors and they do not possess the exit tube characteristic of Plasmodiophoraceae. On the other hand, they agree closely with the endobiotic zoosporangia described by Massee in his work on *P. graminis* (10). While they resemble the epibiotic zoosporangia in their morphology, they are more uniform and seem to follow the host cell shape and are limited by it. These endobiotic zoosporangia are found only in the roots of the host. It seems that the zoospore can infect directly without formation of either the endobiotic or epibiotic sporangia.

Progressive intranuclear divisions of the sporangial nucleus give rise to many nuclei, distributed around a vacuole. Often large aggregations of nucleoli are seen in this vacuole and they appear to discharge chromatin into the surrounding medium (Fig. 1, I). Such an extrusion of the chromatin material from the nucleolus into the surrounding medium has been noted by Kusano in the Synchytriaceae (7). Most of the cytologists dealing with the Chytridiales maintain that the chromatin of the resting nucleus is contained in the densely basophylic nucleolus and is extruded into the nuclear cavity. In *Cladochytrium replicatum*, however, Karling (5) found very little change in the nucleolus during division of the nucleus.

INOCULATIONS ON SEEDLINGS OF A. REPENS AND OTHER GRASSES

The preliminary inoculations were on seedlings of *A. repens* to determine whether there was a differential susceptibility at various ages of the host. Healthy seedlings of *A. repens* 3, 6, 9, 15, and 21 days old (from the

time of plumule emergence) were infected with sporangial suspensions and incubated at 28° C. Results of these experiments indicated a successful and heavy infection in seedlings up to 9 days old and moderate infections in 15- and 21-day-old seedlings. As a further check, epidermis of leaves of various ages was inoculated with sporangia. The results again generally indicated that aging is accompanied by increasing resistance to infection.

The incubation period for expression of the disease is from 10 to 15 days. Corn, teosinte, and lawn grasses associated with *A. repens* in the vicinity of Notre Dame were inoculated with *P. graminis* to determine its host specificity and possible economic significance. *A. repens* and the other grasses mentioned were inoculated also with *P. zeae-maydis*.

The first series of inoculations was in the greenhouse, in small plots in a randomized block design with three replications. Seeds of brome grass (*Bromus inermis*), orchard grass (*Dactylis glomerata*), red top (*Agrostis alba*), timothy (*Phleum pratense*), and quack grass (*Agropyron repens*) were sown in separate pots and the same numbers of seedlings were transplanted to each plot. Teosinte and *Zea mays* were not used because they had been previously unaffected by *P. graminis*.

Preliminary experiments to determine the best method of inoculation were carried out in autumn, 1948, and final experiments laid out in spring, 1949. Sporangial suspensions were sprayed on the seedlings twice on consecutive days to insure infection. Disease-incidence readings were taken on the 10th, 15th, 20th, and 30th day after inoculation. On the 10th day there was no disease; by the 20th day the plots of *A. repens* had brown streaks of the disease but other plants were symptomless. The experiment confirms the field observation that the lawn grasses tested remain free from disease even when in close association with highly diseased *A. repens*.

In a second series of experiments small pots were used and the inoculum was overwintered sporangia of *P. zeae-maydis*. Along with six varieties of *Zea mays* the following grasses were tested for their susceptibility to *P. zeae-maydis*: *Agropyron repens, A. cristatum, Hordeum jubatum, Phleum pratense, Dactylis glomerata, Agrostis alba, Bromus inermis, Sagittaria sanguinalis,* and *Euchloena mexicana*. At the end of 15 days, when the first reading was taken, *Zea mays* had disease symptoms which became clear and fully developed by the end of 30 days. Teosinte was slower in developing the disease symptoms; the other grasses had no infection. The resistance shown by the grasses in both these experiments may be evidence of the existence of physiologic races of the fungus.

DISEASE SURVEYS IN THE NOTRE DAME AREA FOR 1949–1950

The object of these surveys was 1) to determine the method of disease spread in the field and its relationship to environmental factors, and 2) to determine the behavior of the associated grasses to *P. graminis*.

McKinney (11), Horsfall (3), and Horsfall and Barratt (4) have devised grading methods for surveying plant disease. The last, which is an extension of McKinney's, was used in the present instance. This method is

based on the Weber-Fechner law, according to which the human eye distinguishes intensity of disease according to the logarithm of intensity of light stimulus. Plants were graded according to this system and the mean grade determined by the sum of the grade readings divided by the number of readings. The mean percentage was calculated for the entire area and table 2 is a summary of the record along with some pertinent weather data.

For expressing the correlation, an index was formulated which would indicate the degree of favorableness of weather conditions for disease incidence and development. In the table this has been termed favorable index and is arrived at as follows: From laboratory studies it was found that while temperatures between 23° and 31° C. permit sporangial germina-

TABLE 2.—*Disease survey data for 1948–1949*

Year and month	Mean temperature (in ° F.)	Precipitation (inches)	Number of days above 68° F.	Favorable index	Percentage of disease
1948					
September	64.4	3.02	13	0.433	Not recorded
October	49.5	0.93	0	.....	do
November	42.8	2.27	0	.....	do
December	29.4	3.26	0	.....	do
1949					
January	30.0	3.96	0	.....	do
February	28.7	2.89	0	.....	do
March	36.4	2.12	0	.....	do
April	46.1	1.27	0	.....	Trace
May	59.8	3.24	0	0.194	18.5
June	72.0	4.55	23	0.767	47.0
July	76.0	3.53	31	1.000	64.5
August	72.0	3.40	30	0.967	78.4
September	58.6	3.98	1	0.033	55.6
October	56.0	3.03	5	0.161	58.0
November	39.3	2.04	0	.....	14.0
December	32.6	5.01	0	.....	Trace

tion, 29° and 30° C. were optimum. Thus these temperatures in nature would be conducive to the spread of disease. On days with a daily mean of 68° F., at least a portion of the day was ideal for infection. The ratio of such favorable days in the month to the total number of days is termed here the favorable index. The number is expressed as a fraction of unity.

As is evident from the data (Table 2), there is a definite correlation between temperature and disease intensity. Light and precipitation during the months of highest disease incidence varied little and would therefore have the same degree of influence on the disease position. The factor which shows greatest variation and which in the author's opinion is a major determining factor in disease development is temperature. Since the infection precedes symptoms by at least 2–3 weeks, the disease intensity observed in July, for example, is partly a result of the favorable temperature, humidity, and light conditions observed in June.

The numerical favorable index is thought to be accurate for predicting

disease at a given time on the basis of observed weather factors, and by using this index, together with the method of Horsfall and Barratt, a still greater accuracy in disease estimation can be obtained.

DISCUSSION

After the disproval of the sexuality of the turbinate cells of *Urophlyctis*, the difference between that genus and *Physoderma* was reduced to the type of host reaction. Those organisms producing hypertrophies were included in *Urophlyctis* and those producing only discolorations, under *Physoderma*. The finding of hypertrophies in *P. graminis* in the present study and in *P. claytoniana* by Sparrow (16) supports the view of Karling (6), Sparrow (14), and others that the two genera should be merged. Obligate nature of the parasite and its close resemblance to *P. zeae-maydis* in its life cycle suggests a possible relationship. The records of *P. graminis* on various grasses in Europe and the rather restricted parasitism of the present organism to *A. repens* clearly suggest the existence of physiologic races.

The infection of the vascular system by a chytrid is the first report of its kind and the organism is distinctive in the method of dispersal through soil containing disintegrating infected leaves. It seems probable that most new infections occur through the roots as suggested by Massee, since sporangia occurred freely in the roots.

From the descriptions of Massee (10) it is not clear whether he was referring to separate endobiotic zoosporangia or only to developing resting sporangia which are also thin-walled. Structures associated with resting sporangia in the root cortex in the present study appear to be endobiotic zoosporangia.

It is possible that the inhibitory influence of direct sunlight may be actually an effect on enzymatic action. The dormancy of the resting sporangia cannot be regarded entirely as a simple maturation phenomenon, since fresh sporangia collected in spring germinate readily. Since drying delays germination, it is likely that low temperatures coupled with dry conditions prevailing in the autumn might induce impermeability of the exosporangium, which in turn might be an important factor in the resistance of these structures to high and low temperatures. The ligneous nature of the exosporangium supports such a view. They are similar to the overwintering structures of the rusts (teliospores) in color, long viability, and resistance to high and low temperatures.

Pathological anatomy and the host-parasite relationship resemble closely those of other systemic parasites like the smuts, although no definite sori are formed in the present case. Infection of the vascular system in the early stages of development, and of roots and rhizomes, contributes to the characteristic symptoms—wilting and yellowing of the host. Penetration and internal spread of the disease are accomplished presumably by enzymatic dissolution of the cell wall, combined in some cases with mechanical penetration, as indicated by the fineness of the penetrating rhizomycelium.

The reduction in the population of *A. repens* due to the infection by *P. graminis,* noted in a limited area, deserves further consideration. The fact that the fungus inhibits the flowering of the host favors such an investigation.

The disease surveys show a correlation between disease intensity and temperature conditions. This is in agreement with the results of the laboratory experiments with germination of the resting sporangia. The preliminary study on nuclear behavior points to the desirability of further work toward possible elucidation of the interrelationships of the Cladochytriaceae and the Synchytriaceae, both of which contain a large number of phanerogamic parasites.

SUMMARY

The *Physoderma* attacking *Agropyron repens* (L.) Beauv., in Madison, Wisconsin, Chicago, Illinois, and South Bend and Lafayette, Indiana, is determined to be *Physoderma graminis* (Busgen) Fischer. The last three localities are new records for the parasite.

The salient features of the disease symptoms are brown streaks along the leaf veins, erect plant habit, and hypertrophy of the host. Although the symptoms, the systemic and vascular infection, and the flower-inhibiting capacity are distinctive, the life cycle of *P. graminis* closely resembles that of *P. zeae-maydis.*

The resting sporangia are viable over long periods and resist high and low temperatures. Dormancy of these structures can be broken by low-temperature and alternating high- and low-temperature treatment.

Infection occurs in the meristematic regions, rhizomes, and roots. Penetration and extension through the cellulose walls of the host are accompanied by swellings of the rhizomycelia and partial dissolution of the host cell walls. The zoospore might directly establish infection without formation of the epibiotic zoosporangia.

Inoculation experiments indicate that *P. graminis* is restricted to *A. repens,* and that the latter is not a host for *P. zeae-maydis.* Surveys for 1949–1950 show that the peak period of the disease is in mid- and late summer with a slight rise in the autumn. To express the close relationship observed between prevailing temperatures and disease intensity and to predict accurately the disease in the field, a ratio called the "favorable index" is employed.

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LITERATURE CITED

1. CHILDERS, W. R. Record of the occurrence of Physoderma graminis. Science **108**: 484. 1948.
2. CLINTON, G. P. Cladochytrium alismatis. Bot. Gaz. **33**: 49–60. 1902.
3. HORSFALL, J. G. Fungicides and their action. 240 pp. Chronica Botanica Co., Waltham, Mass. 1945.
4. ———, and R. W. BARRATT. An improved grading system for measuring plant diseases. (Abstr.) Phytopath. **35**: 655. 1945.

5. KARLING, J. S. The cytology of the Chytridiales with special reference to Cladochytrium replicatum. Mem. Torr. Bot. Club **19**: 1–9. 1937.
6. ————. The genus Physoderma (Chytridiales). Lloydia **13**: 29–71. 1950.
7. KUSANO, S. Cytology of Synchytrium fulgens Schroet. Jour. Coll. Agr., Univ. Tokyo **10**: 347–388. 1930.
8. LEDINGHAM, G. A. Studies on Polymyxa graminis n. gen., n. sp., a plasmodiophoraceous root parasite of wheat. Canad. Jour. Res. **17**: 38–51. 1939.
9. LEPIK, E. Differential staining of Peronosporaceae. Phytopath. **18**: 869–872. 1928.
10. MASSEE, G. A new grass parasite. Royal Bot. Gard., Kew, Bul. Misc. Inf. **27**: 205–207. 1913.
11. MCKINNEY, H. H. Influence of soil temperature and moisture on infection of wheat seedlings by Helminthosporium sativum. Jour. Agr. Res. [U. S.] **26**: 195–217. 1923.
12. OJERHOLM, E. Multiciliate zoospores in Physoderma zeae-maydis. Bul. Torr. Bot. Club **61**: 13–18. 1934.
13. SPARROW, F. K. Occurrence of true sporangia in the Physoderma disease of corn. Science **79**: 563–564. 1934.
14. ————. Aquatic Phycomycetes, exclusive of the Saprolegniaceae and Pythium. 785 pp. Univ. Mich. Press. 1943.
15. ————. Observations on chytridiaceous parasites of Phanerogams. II. A preliminary study of the occurrence of ephemeral sporangia in the Physoderma disease of maize. Amer. Jour. Bot. **34**: 94–97. 1947.
16. ————. Observations on chytridiaceous parasites of Phanerogams. III. Physoderma Claytoniana and an associated parasite. Amer. Jour. Bot. **34**: 325–329. 1947.
17. THIRUMALACHAR, M. J. A method of germinating and staining teleutospores. Jour. Ind. Bot. Soc. **19**: 71–75. 1940.
18. ————, and J. G. DICKSON. A Physoderma disease of quack grass. Phytopath. **37**: 885–888. 1947.
19. TISDALE, W. H. Physoderma disease of corn. Jour. Agr. Res. [U. S.] **16**: 137–154. 1919.

EFFECT OF VARIOUS CHEMICALS ON THE NATURAL HEALING OF FRESHLY CUT POTATO SETS[1]

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In a recent paper (6) it was shown by the author that within 21 days, at 61° F., soil-borne *Fusarium coeruleum* (Lib.) Sacc. was capable of destroying untreated freshly cut potato sets, especially when in a dry soil. It was also shown that, when applied to the surface of freshly cut sets, certain chemicals, notably Spergon, afforded them almost complete protection while others, now commonly used to treat whole seed tubers, were more or less ineffective. The merits of treating freshly cut sets with a chemical effective against certain soil- and tuber-borne plant pathogens, instead of treating the whole tubers and then cutting them, were emphasized.

In order to assess the usefulness of a suitable chemical treatment of cut potato sets, one should determine the effect of the chemical on the natural healing processes at the temperature and relative humidity at which the sets are stored or planted, as well as the fungicidal or protective effectiveness of the chemical employed. In Alberta, and in many other areas, when the cut sets are stored or planted in the field, the temperature may often vary between 51° and 61° F.; also the relative humidity in storage is often low, except where the sets are in large piles or in sacks. Therefore, in this study, the primary purpose was to compare the effect of various chemicals on the natural healing processes of freshly cut potato sets, when they are buried in a soil of approximately optimum moisture content and at 61° F.

LITERATURE REVIEW

The importance of a continuous layer of naturally suberized or "blocked" cells on the cut surface, and of a well-formed and continuous wound periderm layer below this outside blocked surface layer, in resisting the entrance of plant pathogens is well recognized by Appel (1), Priestley and Woffenden (4), Artschwager (2), Leach (3), and others. Normally the first and essential step in this natural healing process is the suberization of a layer of intact cells on the cut surface (5). Therefore any physical, chemical, or biological factor that can speed up the formation of a continuous outside suberized layer may become very important in the exclusion of fungal parasites, as well as in the subsequent development of wound periderm layers below. Weiss, Lauritzen, and Brierley (7) found that, at 61° F., the cells on the cut surface require 3 days to heal well enough to

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successfully resist penetration by *F. coeruleum*. This fact is very important, because it probably explains why untreated, freshly cut sets planted in a soil held at 61° F. and infested with *F. coeruleum*, were completely rotted in 21 days (6). Presumably an effective suberized barrier was not formed soon enough to prevent infection.

MATERIALS AND METHODS

Experiments to test the effect of various chemicals on the natural healing processes of freshly cut potato sets were carried out in unsterilized black Edmonton virgin loam, maintained at approximately optimum water content and at 61° F. The potato variety used was Warba (or Netted Gem). The sets were cut from the tubers with a vegetable baller and mixed thoroughly to ensure a representative sample, after which they were treated with the test chemical and immediately buried deeply in the test soil.

At stated periods, indicated in tables 1 and 2, a representative sample consisting of the sets of each variety was removed from the soil and held briefly in a refrigerator at 40° F., until freehand sections could be cut from them. These sections were mounted on microscope slides and stained for suberin with Sudan III, examined under the microscope, and rated for intensity and continuity of the natural healing processes as affected by the various chemicals.

The different chemicals used in treating the freshly cut sets were: Spergon (tetrachloro-para-benzoquinone), 1 per cent agitated liquid; Semesan Bel (nitro-phenol mercury plus chlorophenol mercury), strength according to directions on package, liquid; Ceresan (5 per cent ethyl mercury phosphate), 1 per cent agitated liquid; mercuric chloride solution, strength 1:1,000 (normal and acidified); calcium hydrate (commercial), dust; sulphur, dust; Fermate (ferric dimethyl dithiocarbamate), dust. The sets were dipped for 1 min. in the liquid treatments and agitated.

EXPERIMENTAL WORK

The rate of natural healing in freshly cut sets was studied at 61° F. where the sets were treated with the various chemicals; at 61° and 78° where the sets were not treated with chemicals; and finally where the rate of healing in healthy sets was compared with that in sets infected with the leaf-roll virus. The results of each experiment are presented in the sequence just mentioned.

Effect of Chemicals on the Natural Healing of Cut Sets at 61° F.

Blocking of cells on cut surface. At the end of 3 days the development of the "blocking" process of the intact surface cells of the cut sets treated with the various chemicals was rated and expressed in percentage (Table 1). The blocking process, which apparently began soon after the sets had been cut, developed rapidly, except in those treated with Ceresan. This chemical blackened the outer layer of uncut cells and delayed their suberization.

The other mercurials, namely, mercuric chloride and Semesan Bel, clearly retarded the blocking process. Sulphur and Fermate perceptibly delayed the blocking process more than did Spergon, but certainly less than the mercurials. Blocking occurred sooner and seemed more pronounced in the sets treated with calcium hydrate than in those treated with Spergon.

Wound periderm formation. At the end of 10 days, there was no evidence of periderm layers in the untreated control, but three definite layers had formed in those treated with calcium hydrate and one layer in those treated with Spergon (Table 1). Apparently periderm layers were still

TABLE 1.—*Effect of various chemicals on the rate of natural healing in freshly cut potato sets, when buried in virgin Edmonton black loam maintained at optimum water content and at 61° F.*

Chemical treatment	Percentage of blocking[a] (3 days)	Approximate numbers of wound periderm layers—percentage rating of tubers		
		10 days	15 days	21 days
None	90	0—0	4—80	4—100
Spergon	100	1—5	4—80	4—100
Calcium hydrate	100	3—50	6—100	6—100
Fermate	60	0—0	4—70	4—100
Sulphur	50	0—0	4—60	4—100
Semesan Bel	50	0—0	3—50	4—100
Mercuric chloride (1: 1,000)	50	0—0	3—50	4—100
Mercuric chloride[b] (acidified)	50	0—0	3—50	4—100
Ceresan	?	0—0	2—30	4—100

[a] Suberization of intact cells on cut surface.
[b] Ten ml. hydrochloric acid per litre.

absent in the Fermate-treated and sulphur-treated sets, and also absent in those treated with the mercurials.

At the end of 15 days, the wound cork layers were again very definite in the sets treated with calcium hydrate, the layers having increased to approximately six. There were about four layers in the sets of the control, and in those treated with Spergon, Fermate, and sulphur; and about three layers in those treated with mercuric chloride or Semesan Bel. Only two layers had formed in the sets treated with Ceresan.

At the end of 21 days, about the same number of layers of wound periderm was present in the sets of the various treatments. Apparently these layers were continuous through the vascular strands at this date, in contrast to discontinuous layers at the end of the 10- and 15-day periods.

Effect of Temperature on Natural Healing

Priestley and Woffenden (4) found that, in a humid environment, layers of wound periderm formed in about 48 hr. at 78° F., but at 61° they did not appear until about 12 days. These authors and others found that dry conditions and poor aeration were unfavorable to the healing process.

Obviously, when the healing process is slow, especially across the vas-

cular strands, certain parasites may enter the set and arrest or delay the healing process. Therefore, a small experiment was carried out to show the difference in the rate of natural healing in cut sets held at 61° and 78°. The varieties chosen for the purpose were Warba (early), and Katahdin (late) (Table 2).

At the end of 2 days, the blocking of the cells on the cut surface at 61° F. was definite, although slight, in the sets of both varieties, but good at 78°. Wound periderm was absent at 61°, but fair at 78°. At the end of 5 days,

TABLE 2.—*Rate of formation of wound cork periderm in freshly cut potato sets in soil of optimum moisture at 61° and 78° F.*

Days	Temperature (° F.)	Variety	Soil		Air	
			Blocking	Wound periderm	Blocking	Wound periderm
2	61	Warba	Slight	0	Slight	0
2	61	Katahdin	Slight	0	Slight	0
2	78	Warba	Good	Fair	Good	Fair
2	78	Katahdin	Good	Fair	Good	Fair
5	78	Warba	.....	Excellent	.....	Excellent
5	78	Katahdin	.....	Excellent	.....	Excellent

wound periderm formation at 78° was heavy for both varieties, despite an occasional inconsistency in the sets from different tubers of the same variety.

Effect of Leaf-roll Virus on Rate of Natural Healing of Freshly Cut Potato Sets

The widely recognized tendency for potato tubers or sets infected with leaf-roll virus to resist decay in the soil has been mentioned frequently, but apparently no one has determined whether or not such resistance is due to a more rapid natural healing process characteristic of tubers infected with leaf roll. Accordingly, a separate experiment was designed for the purpose indicated, and essentially the same methods as already described were employed. The only difference was that in one series the sets were cut from infected tubers, and in the other from healthy tubers of the same variety, namely, Netted Gem. The freshly cut sets were untreated and planted in a soil maintained at optimum moisture content and at 61° F. The degree of blocking at 3 and 5 days was equally good in both the healthy and the leaf-roll sets. The formation of wound periderm was also equally good at the end of 12 days. Therefore, it appears that the greater resistance to decay in potatoes infected with the leaf-roll virus is due not to a better healing process but to some other factor.

DISCUSSION

The primary purpose of this investigation was to determine the effects of different chemicals on the rate of natural healing in freshly cut potato sets buried in a soil of optimum moisture and at 61° F.

With regard to the untreated sets, the results of this experiment are in general agreement with those reported by previous investigators (1, 2, 3, 4). The suberization of the intact cells of the cut surface, which apparently begins soon after the cut is made, was well advanced at the end of 3 days, but still incomplete. However, at the end of 10 days apparently no layers of wound periderm had been formed, but by the 15th day three or more layers were obvious. Often the layers of wound periderm were delayed temporarily, and probably the external suberization as well. Naturally, this condition would invite infection by parasitic fungi and bacteria, which could delay or prevent further healing, and also permit their growth in the tissue. The greatly accelerated rate of healing at 78° F. compared to that at 61° was verified.

Regarding the effect of the chemicals on the natural healing processes at 61° F., the experimental results of the present study show that in moist soil calcium hydrate on the cut potato sets obviously stimulated the incidence of suberization of the cells of the cut surface, and also of wound periderm. Priestley and Woffenden (5) found in their experiments that neither caustic nor slaked lime aided the normal deposit of suberin and probably retarded it because of their drying effect on the cut surface in ordinary farm practice. In these experiments, Spergon also appeared to stimulate the natural healing processes, whereas sulphur, and probably Fermate, were more or less detrimental. The mercurials, especially Ceresan, definitely delayed early suberization of the cut surface, but the detrimental effect on wound periderm formation had disappeared by the end of 21 days. The nature of the influence of each chemical used on the healing process was not determined. Priestley and Woffenden (4) pointed out that the starch grains in the cells adjacent to the cut surface break down, free fatty acids are formed, suberin is deposited on the walls of each blocked cell, and the cellulose walls of these cells also become impregnated. Observational evidence from the present study is in agreement with the results just mentioned.

The tendency of certain chemicals to retard or favor the natural healing process, particularly during the first 3 days, no doubt has an important practical bearing on whether or not the cut potato sets become infected by important soil-borne parasites. Although both calcium hydrate and Spergon undoubtedly favored the healing processes, only the latter one provides (6) the necessary protection against invasion by *F. coeruleum*. Also, despite the well-known fungicidal value of the different mercurials used, they failed to protect the sets against invasion and also retarded the natural healing processes.

The foregoing results suggest that the ideal chemical for treating freshly cut potato sets would be one that offered effective protection against parasitic fungi and bacteria while in storage or in the soil, and also favored the healing process. Spergon appears to possess both of these desirable qualities in so far as *F. coeruleum* is concerned. Other untried chemicals

may be even better in protecting the cut sets against the various parasites, including *Erwinia carotovora* (Jones) Holland, *Pythium ultimum* Trow., and *Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. & Burkh., and also in destroying *Rhizoctonia solani* Kühn and *Streptomyces scabies* (Thaxt.) Waksman & Henrici on the uncut surface of the tubers.

At 78° F. healing of the cut sets is obviously rapid and fairly effective against infection, but at 61° F. or lower, which temperature is common at planting time, the healing is too slow, and, therefore, the sets should be protected by a chemical treatment.

SUMMARY

The effect of various chemicals on the natural healing processes of freshly cut potato sets, when held in moist soil at 61° F., was studied. Calcium hydrate accelerated the incidence of external suberization and layers of wound periderm, and Spergon also favored it. Fermate and sulphur delayed it slightly. Mercuric chloride solution (standard and acidified), and Semesan Bel obviously delayed it during the first 3 days, and the effect of Ceresan was very detrimental. The differential effect of the chemicals was most evident during the early suberization of the cut surface and up to 10 days, but it became indefinite soon after 15 days.

Cut sets infected with leaf roll healed as quickly as healthy ones. The healing at 61° was slow compared to that at 78° F.

The treatment of cut potato sets with an effective fungicide, also favorable to natural healing, is suggested to replace the present practice of disinfecting whole tubers.

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LITERATURE CITED

1. APPEL, O. Zur Kenntnis des Wundverschlusses bei den Kartoffeln. Ber. Deut. Bot. Gesell. **24**: 118–122. 1906.
2. ARTSCHWAGER, ERNST. Wound periderm formation in the potato as affected by temperature and humidity. Jour. Agr. Res. [U.S.] **35**: 995–1000. 1927.
3. LEACH, J. G. Blackleg disease of potatoes in Minnesota. Minn. Agr. Exp. Sta. Tech. Bul. 76. 1931.
4. PRIESTLEY, J. H., and L. M. WOFFENDEN. Physiological studies in plant anatomy. V. Causal factors in cork formation. New Phytologist **21**: 252–268. 1922.
5. ————, and ————. Healing of wounds in potato tubers and their propagation. Ann. Appl. Biol. **10**: 96–115. 1923.
6. SANFORD, G. B. Prevention of early decay of cut potato sets by chemical treatment. Sci. Agr. **29**: 345–350. 1949.
7. WEISS, F., J. I. LAURITZEN, and P. BRIERLEY. Factors in the inception and development of Fusarium rot in stored potatoes. U. S. Dept. Agr., Tech. Bul. 62. 1928.

MOSAIC RESISTANCE IN CABBAGE

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In a previous paper,[2] mosaic of cabbage (*Brassica oleracea* var. *capitata* L.) in midwestern and northwestern areas of the United States was shown to be incited by the interaction of a strain of turnip virus 1, which produces a mottle symptom, and cauliflower virus 1, which produces veinclearing and chlorosis. These viruses were designated respectively as cabbage viruses A and B. In a later paper[3] cabbage varieties were shown to differ markedly in their reactions to mosaic. In the course of the program of breeding cabbage for resistance to yellows, the possibility of introducing mosaic resistance into various yellows-resistant types was explored. The present paper is a report of studies of selection for mosaic resistance within yellows-resistant lines and of the inheritance of mosaic resistance in crosses of highly resistant and susceptible parents. The investigation began in 1942 but was conducted for the most part from 1946 to 1950, inclusive.

METHODS AND MATERIALS

Cabbage seedlings were grown in greenhouse flats, where they were inoculated in the second true leaf stage with a mixture of cabbage viruses A and B. Following inoculation, the plants were removed to cold frames where they were kept until transplanted to the field. In the field plots rows of commercial varieties, inoculated and noninoculated, were set at frequent intervals as controls. The same commercial varieties were repeated each year to check one season against another. By midseason practically 100 per cent of the plants in healthy control rows were infected by aphid transmission. Detailed notes were taken each year during the week of September 10 and again one month later. The second series of notes was necessitated because certain symptoms developed only very late in the season. Each plant was rated for symptom severity and placed in one of five disease classes, 0, 1, 2, 3, and 4, depending upon whether symptoms were absent, slight, moderate, severe, or very severe, respectively. In calculating the disease index, the number of plants in each class was multiplied by the class number, the sum of the products was multiplied by 100, and the result was divided by 4 times the total number of plants. Thus if all the plants were in class 0, 1, 2, 3, or 4 the index value would be 0, 25, 50, 75, or 100, respectively. Two distinct categories of symptoms were re-

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[2] Walker, J. C., Francis LeBeau, and Glenn S. Pound. Viruses associated with cabbage mosaic. Jour. Agr. Res. [U.S.] 70: 379–404. 1945.

[3] Pound, Glenn S. Reactions of cabbage varieties to mosaic viruses. Jour. Agr. Res. [U.S.] 75: 19–30. 1947.

corded and an index was calculated for each. The first consisted of mottle, leaf distortion, and plant stunting. The second was a general chlorosis which appeared in the cool late part of the season. It was incited primarily by the B virus. Resistance to the two syndromes is referred to as mottle resistance and chlorosis resistance, respectively.

EXPERIMENTAL RESULTS

Selection within yellows-resistant lines. It became obvious early that discontinuous classes of resistant and susceptible plants did not occur. This necessitated the measuring of resistance by classifying plants in the five arbitrary classes already mentioned. The variance in environment affected the expression of resistance somewhat from year to year. Very susceptible progenies were quite consistent in showing severe symptoms regardless of summer temperature, but resistant progenies tended to show less severe symptoms at low temperatures, about 16° C., than at high temperatures, around 28°. In table 1 are shown the index values over a period of years

TABLE 1.—*Increase in mosaic resistance in cabbage by selection*

Selection line or control variety	Disease index of selections made in year shown					
	1942	1946	1947	1948	1949	1950
25-10	20.3	.....	.....	.....	.....	.....
25-38	35.5	.....	.....	.....	.....	.....
2549P (derived from 25-10 × 25-38)	.....	13.9[d]	16.3	15.3	19.0	18.2
2549N (derived from 25-38 × 25-15)[a]	.....	27.1[d]	.....	35.4	32.7	38.8
Wisconsin All Seasons (control)[b]	45.7	45.8	51.5	44.2	44.8	50.5
83-557 (Wisconsin Hollander Selection)	.....	52.5	46.9	39.0	21.5	15.6
Ferry's Hollander (control)[c]	.....	70.5	73.0	76.0	75.0	77.2
Improved Wisconsin Ballhead (control)[c]	.....	48.7	48.1	44.0	.....	54.0
Wisconsin Hollander (control)[c]	51.3	57.3	55.0	55.3	60.9	52.3

[a] 25-15 was not included in the 1942 test.

[b] This is the parent stock from which the parents of 2549P and 2549N were derived. The same seed lot was grown each year (1942–1946).

[c] The same seed lot was grown each year (1942–1946) for these varieties.

[d] Selections from crosses made in 1942 were continued during 1943, 1944, and 1945, but disease indices were not calculated.

for a very susceptible variety, Ferry's Hollander, and three somewhat less susceptible varieties, Improved Wisconsin Ballhead, Wisconsin Hollander, and Wisconsin All Seasons. The last three varieties are also resistant to yellows.

The first studies were concerned with improvement of resistance by selection within established yellows-resistant varieties. In 1942 nearly 100 progenies derived from Wisconsin All Seasons were tested for mosaic resistance. Two of these, 25-10 and 25-38, and 25-15, a sister progeny not tested in 1942, were selected for further study. The indices in 1942 of 25-10, 25-38, and the parent progeny are shown in table 1. Plants from progenies 25-10 and 25-38 were crossed in the greenhouse in the winter of 1942–1943 and selection of resistant individuals was made in succeeding years, the

plants in each case being self-pollinated. Indices were not recorded in 1943, 1944, and 1945; but after three years of selection the line (2549P) showed an index of 13.9 in 1946, and in the four succeeding years further inbreeding did not result in any reduction or in any appreciable change in the disease index. In another line, 2549N, derived from a cross of plants from progenies 25-38 and 25-15 and carried on simultaneously with 2549P, an index of 27.1 was recorded in 1946. Again further inbreeding and selection did not lower the disease index. As the two lines became stabilized, 2549P was decidedly more resistant than 2549N. In another line, 83-557, selection for resistance was carried out within Wisconsin Hollander beginning in 1945, with the first progeny test in 1946. A steady increase in resistance with each succeeding generation up to 1950 was found as shown by the decline in disease index. In lines 2549P and 2549N, on the other hand, respective levels of resistance appear to have been established at the end of three generations of selection.

These results indicated that resistance was a quantitative character. This would account for the lack of discontinuous classes of disease and would explain the fact that in different selection lines resistance becomes stabilized at different levels. In order to explore further into the nature of inheritance of resistance to mosaic, crosses were made between stabilized highly resistant and very susceptible lines.

Inheritance of Resistance: Mottle-resistant × *mottle-susceptible.* In table 2 are the results from crosses between a plant (25-736) from a resistant line of Wisconsin All Seasons and a plant (83-122) from a rather highly susceptible line of Wisconsin Ballhead. Progenies of a cross between 25-736 and 83-117, a sister plant to 83-122 (data not presented in tabular form), were similarly studied. The progenies from the parent plants secured by selfing or sib-mating were tested in each case. In the tests of the parent lines and the F_1 progenies it was found that the two susceptible plants had almost identical disease indices, but most of the population was in class 3 in the case of 83-122 (Table 2), whereas in 83-117 it was distributed over classes 1 to 4. The index of the F_1 progeny from each cross was between those of the parents, but in each cross it was distinctly lower and resistance was thus higher than the mean of the two parents. No plants in either F_1 progeny were placed in the two highest disease classes where a majority of those of the susceptible parent progenies were found. This showed substantial but incomplete dominance of resistance. In the F_2 progeny from each cross the disease index was close to the arithmetic mean of the indices of the two parents. The F_2 populations were distributed over the five disease classes with a skewness in the distribution curve in the direction of the more resistant classes.

In the F_3 progeny tests from plants taken at random from noninfected F_2 populations of cross 25-736 × 83-117, only one of 11 closely approached the susceptible parent in population distribution and disease index. All others were intermediate between the two parents in distribution and none

approached that of the resistant parent. In the same analysis of F_3 progenies of cross 25-736 × 83-122 (Table 2), where most of the population of the susceptible parent was in class 3 and where there was evidence of transgressive segregation in the F_2, eight of 14 progenies showed evidence of transgressive segregation in that plants in class 4 were recorded. As in cross 25-736 × 83-117, no progeny approached the resistant parent in population distribution. Undue emphasis on transgressive segregation does not seem to be justified, however, since in the 1947 test of 83-122, the susceptible parent line, the play of environment may have excluded some plants from class 4 which might have been found to belong in that class if it had been included in the 1949 tests.

TABLE 2.—*Frequency distribution of plants of 25-736 (resistant) and 83-122 (susceptible) and of progenies of their cross*

Progeny	Year grown, and generation	Number of plants in mosaic severity class					Disease index
		0	1	2	3	4	
25-736	1947–P_1	18	14	3	0	0	14.3
83-122	1947–P_1	0	1	2	37	0	72.5
25-736 × 83-122	1947–F_1	5	34	6	0	0	25.6
	1948[a]–F_2	5	92	125	49	7	46.5
83-452	1949[a]–F_3	1	13	32	1	0	42.6
83-453	F_3	0	8	15	3	0	45.2
83-454	F_3	0	7	8	24	8	67.6
83-455	F_3	0	20	4	0	0	29.2
83-457	F_3	0	1	23	15	2	61.0
83-458	F_3	0	20	19	2	0	39.0
83-460	F_3	0	1	8	24	16	78.1
83-463	F_3	0	1	15	18	9	70.3
83-465	F_3	1	9	16	10	3	42.3
83-466	F_3	0	20	12	3	0	37.9
83-467	F_3	0	4	25	2	6	56.8
83-468	F_3	0	0	5	16	4	74.0
83-469	F_3	0	0	9	22	20	80.4
83-471	F_3	0	8	31	2	0	46.3

[a] The F_2 population consisted of seed of sib matings of F_1 plants. The F_3 population consisted of seed of self-pollinated F_2 plants.

The results of a third cross are in table 3. The resistant parent progeny had a slightly higher index than that of the one in table 2; the susceptible parent was much more nearly homozygous for the susceptible character than susceptible parents in the two previous crosses. In the F_1 progeny the resistance of 25-824 was apparently completely dominant as indicated by the disease index and the distribution of population in disease classes. Representative plants from the parent progenies and from the F_1 progeny are shown in figure 1. In the F_2 progeny there was again a dispersion of the population over nearly the full range of the disease classes of both parents and a skewness in the direction of the resistant parent. Fourteen F_3 progenies were tested; seven of these (25-68s to 25-75s, inclusive) were selected at random from uninoculated F_2 plants; the remainder were from

TABLE 3—*Frequency distribution of plants of 25-824 (resistant) and 83-367 (susceptible) and of progenies of their cross*

Progeny	Year grown, and generation	Number of plants in mosaic severity class					Disease index
		0	1	2	3	4	
25-824	1948–P_1	5	33	6	0	0	25.6
83-367	P_2	0	0	0	0	41	100.0
25-824 × 83-367	1948–F_1	2	18	5	0	0	28.0
	1949–F_2	0	41	53	5	2	42.1
25-68s	1950–F_3	0	32	18	0	0	34.0
25-69s	F_3	0	24	19	0	0	34.9
25-70s	F_3	0	23	24	3	0	40.0
25-71s	F_3	1	26	23	0	0	36.0
25-72s	F_3	1	16	20	1	0	38.8
25-73s	F_3	0	50	4	0	0	26.9
25-75s	F_3	0	40	10	0	0	30.0
25-77s	F_3	0	42	8	0	0	29.0
25-78s	F_3	1	38	11	0	0	30.0
25-79s	F_3	0	28	22	0	0	36.0
25-80s	F_3	0	24	26	0	0	38.0
25-81s	F_3	0	2	21	18	4	63.3
25-82s	F_3	0	45	31	0	0	35.2
25-83s	F_3	0	25	3	0	0	27.7

infected F_2 plants. All of these progenies were intermediate between the two parents in population distribution.

In a fourth cross in the series (data not given), resistance appeared to be slightly more dominant in the F_1 than in the first two crosses. Skewness in the direction of the resistant parent again appeared in the F_2 distribution. All of the eight F_3 progenies tested were intermediate between the parents in population and distribution; seven of them showed skewness in the direction of the resistant parent.

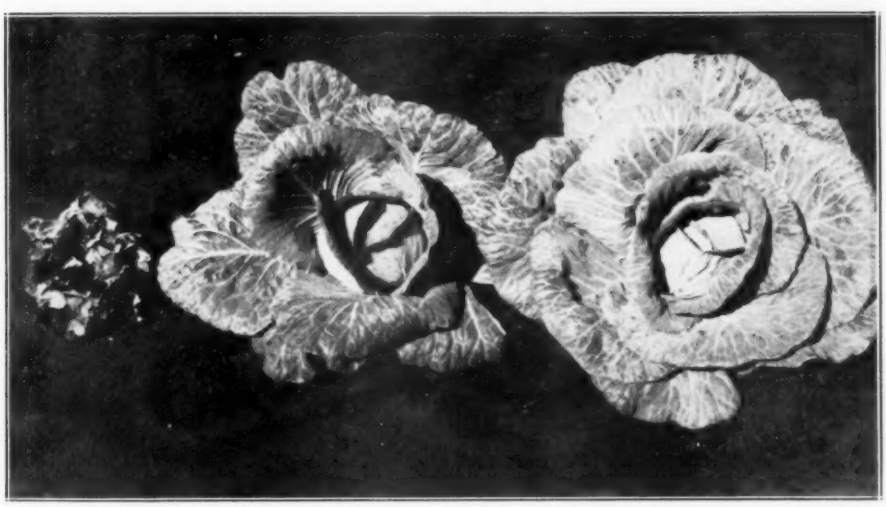

FIG. 1. Mosaic resistance in cabbage. Left, mottle-susceptible 83-367; right, mottle-resistant 25-824; center, representative F_1 plant of the cross 25-824 × 83-367. Note resistance of the F_1 progeny.

When two parents from resistant lines of Wisconsin All Seasons were crossed (data not given), F_1, F_2, and F_3 progenies all coincided closely with both parent progenies in disease index and in frequency distribution.

Inheritance of resistance: Mottle-resistant chlorotic × mottle-resistant nonchlorotic. Virus B of the cabbage mosaic complex produces in late season a chlorosis which greatly resembles a mineral deficiency chlorosis (Fig. 2). This symptom occurs independently of the mottle induced by virus A. It does not appear in Wisconsin until relatively cool temperatures prevail in the fall, in association with approaching maturity. Although the symptom develops severely at this time it does not cause any noticeable

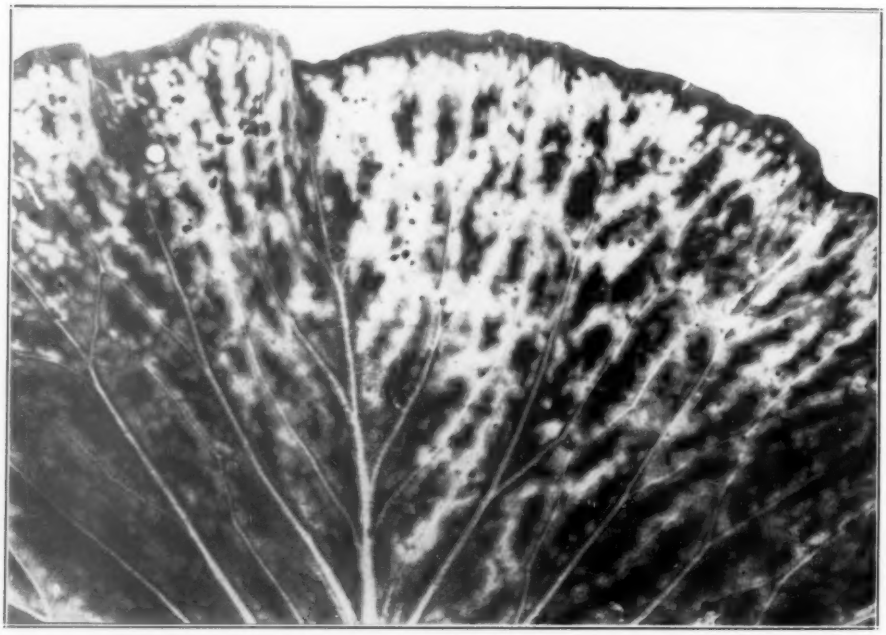

FIG. 2. Late-season chlorosis induced by the B virus component of cabbage mosaic.

yield reduction. The symptom has been produced, however, on young plants in the greenhouse at low temperatures.

Mottle-resistant, chlorotic-susceptible plants were crossed with plants from mottle-resistant lines which developed little or no chlorosis. In each of two crosses (Table 4) the F_1 was somewhat intermediate between the parents but was much nearer the nonchlorotic parent. In the F_2 populations, segregation occurred and the overall index of chlorosis increased considerably over that of the F_1, although it was still intermediate between the indices of the parents. Certain F_2 plants of the cross 25-814 × 25-864 were recorded as to their reaction. Self pollinated seeds from these plants were tested in 1950. By selection for resistance or susceptibility in the F_2, markedly divergent lines were obtained in the F_3 (Table 4). Heads from F_2 population of the cross 25-814 × 83-366 were selected at random in a

virus-free plot. Of seven resultant F_3 progenies, one resembled the susceptible parent progeny in disease index and distribution among disease classes and four resembled the resistant parent, while two were intermediate. These data would indicate that this chlorotic character, as well as the mottle character, is inherited quantitatively.

SUMMARY AND CONCLUSIONS

A relatively high degree of resistance to mosaic can be developed within most if not all cabbage varietal types by selection. Highly resistant lines have been developed by selection within Wisconsin All Seasons, Wisconsin Ballhead, and Wisconsin Hollander, each of which contains single-gene resistance to Fusarium yellows.

TABLE 4.—*Frequency distribution of plants of 25-814 (susceptible), 25-864 (resistant), and 83-366 (resistant) and of progenies of the crosses, 25-814 × 25-864 and 25-814 × 83-366—B chlorosis*

Progeny	Year grown, and generation	Number of plants in chlorosis severity class					Disease index
		0	1	2	3	4	
25-814	1948–P	12	23	13	2	0	27.5
25-864	P	32	3	0	0	0	2.1
83-366	P	51	1	0	0	0	0.5
25-814 × 25-864	1948–F_1	36	9	1	0	0	6.0
	1949–F_2	57	53	21	0	0	18.1
25-54[a]	1950–F_3	3	34	8	0	0	27.8
25-56[b]	F_3	41	11	2	0	0	6.9
25-57[a]	F_3	3	32	14	0	0	30.6
25-814 × 83-366	1948–F_1	46	3	0	0	0	1.5
	1949–F_2	56	25	8	0	0	11.5
25-58	1950–F_3	43	5	0	0	0	2.6
25-59	F_3	38	7	4	0	0	7.7
25-60	F_3	46	1	0	0	0	0.5
25-64	F_3	50	0	0	0	0	0.0
25-65	F_3	48	3	2	0	0	3.3
25-66	F_3	19	6	5	0	0	13.3
25-67	F_3	10	32	12	0	0	25.9

[a] Parent plants of 25-54 and 25-57 showed chlorosis in 1949.
[b] Parent plant of 25-56 showed no chlorosis in 1949.

Resistance to the mottle phase of the disease was incompletely dominant. The F_1 progenies of mottle-resistant × mottle-susceptible crosses resembled closely the resistant parent progeny in disease index and in population distribution in disease classes. The F_2 progenies were intermediate between the parent progenies in disease index, but the distribution in disease classes showed a skewness in the direction of that of the resistant parent. It was not possible to test enough F_3 progenies upon which to base a factorial interpretation of inheritance. In most of the F_3 progenies of each cross there was noticeable skewness of distribution in the direction of that of the resistant parent, while there was an occasional F_3 progeny with skewness in the opposite direction. These data would indicate that resistance is polygenic but that it is controlled by relatively few gene pairs.

Although the parents were products of inbreeding and were quite uniform in their reaction, it is not probable that they had been inbred to the extent that they were homozygous for all the gene pairs influencing resistance. Resistance is apparently fixed approximately by relatively few generations of inbreeding and selection and a point is reached beyond which little or no further increase is obtained.

In crosses between resistant plants derived from different lines of Wisconsin All Seasons, resistance was maintained in the F_1, F_2, and F_3 progenies. In crosses between resistant Wisconsin All Seasons and susceptible Wisconsin Ballhead, selections among segregating F_2 populations gave rise to resistant lines with Ballhead characters as well as susceptible lines with All Seasons characters. This indicates that resistant lines can be secured readily by hybridization of resistant and susceptible varieties followed by selection.

Resistance to the chlorosis symptom incited by the B virus also was incompletely dominant and, although the evidence is fragmentary, this resistance appears to be inherited quantitatively. Resistance to this symptom is independent of that for mottling since mottle-resistant but chlorosis-susceptible lines have been obtained. This does not preclude possible linkage of genes for reactions to mottle and chlorosis.

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TOMATO ROSETTE, A SEVERE DISEASE CAUSED BY A STRAIN OF TOBACCO MOSAIC VIRUS[1]

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A serious disease of tomato (*Lycopersicon esculentum* Mill.), here designated as tomato rosette, was observed in various sections of Virginia during the 1949 and 1950 growing seasons. Because of the highly infectious nature of the disease and its effect on the crop, it constitutes a serious threat to the growing of tomatoes in regions in which it is at all prevalent. This paper records a description of the disease, together with experimental evidence for identification of its causal agent as a strain of tobacco mosaic virus.

DESCRIPTION OF THE DISEASE

Tomato rosette was first observed in 1949, and again in 1950, on a truck farm near Roanoke, Virginia. It was also observed on various farms in the vicinity; in most cases, the farmers had bought seedling plants from the truck farm on which the disease was first seen. During the 1950 season, the same or a similar disease was observed in various other parts of the State, including the Norfolk area.

The most marked characteristic of the disease on tomato is extreme malformation of the foliage accompanied by severe stunting. The degree of malformation varies with different plants and, presumably, with different growing conditions. In general, leaves are reduced in size and variously curled, twisted, and narrowed. In extreme cases, leaflets appear as tendrils, most of the lamina being absent, and, when many such tendrils occur together, give the plant a witch's-broom effect. Plants thus affected resemble those accidentally, or otherwise, treated with 2,4-dichlorophenoxyacetic acid (2,4-D). Less severely malformed leaves may be tendrillike only at their tips, the basal portions retaining much of the lamina but being narrowed and misshapen (Fig. 1, B). In still other instances, entire leaves may be fernlike (Fig. 1, C). Leaves on severely affected plants, if not tendrillike, are branched, rolled, or cupped and give the plant a rosetted appearance (Fig. 1, A). These symptoms, although not so common as the shoestring symptoms, led to the choice of the name rosette for the disease. The shoestringing or tendrillike effect on leaves is characteristic of the disease and is of diagnostic value. However, it appears late in the course of the infection, after the plant has attained considerable size.

The mottling on affected leaves is not usually pronounced but otherwise

[1] Contribution from the Plant Virus Laboratory of the University of Pittsburgh.

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is similar to that caused by mild strains of tobacco mosaic virus, being green rather than yellow. During onset of symptoms it is pronounced and is accompanied by mild curling and distortion of leaves. In this stage, rosette is indistinguishable from the disease produced by mild strains of tobacco mosaic virus. In the greenhouse, experimentally infected plants produced flowers but seldom set fruit.

TRANSMISSION

The symptoms of tomato rosette as first observed in the field led to the supposition that it might be caused by a virus of the yellows group. Therefore, first attempts at transmission were made by grafting small pieces of diseased tissue into the stems of young tomato plants. Each such attempt

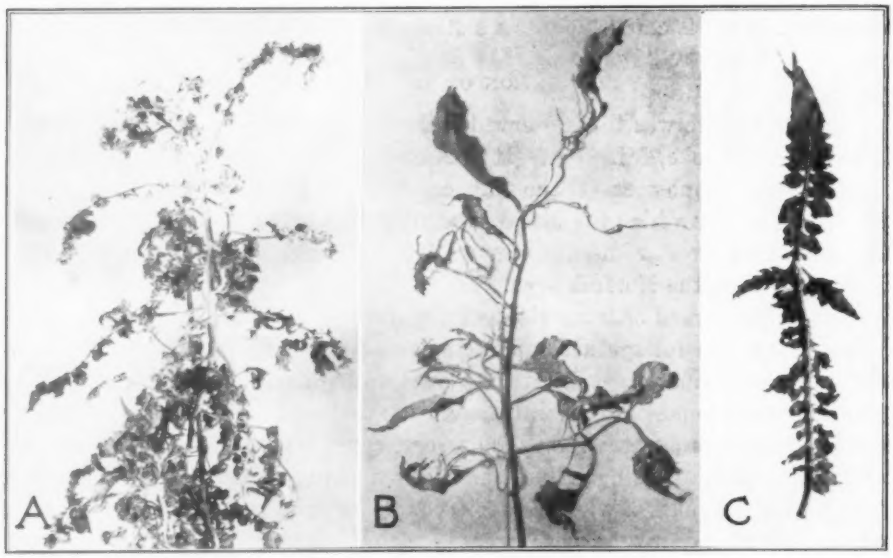

FIG. 1. Tomato plants experimentally infected with tomato rosette virus: A, rosette symptoms; B, shoestring symptoms; C, fern-leaf symptoms.

resulted in transmission. Subsequently, transmission was readily obtained by rubbing the sap from a diseased leaf onto leaves of a healthy plant. In this way the virus has been transmitted from tomato to tomato, to tobacco (*Nicotiana tabacum* L.), and to other plants. Experimentally infected tomato plants have developed tendrillike leaves and other symptoms like those seen on field-grown plants. Symptoms on these experimentally infected plants were, however, somewhat less severe than those observed in the field. Individual tomato plants have shown differences in their response to infection. Those infected late in the fall were slower in producing the shoestring effect. These symptoms have appeared as early as 3 weeks after inoculation or have been delayed for 8 weeks or longer.

During the winter months of 1949–1950 several sets of tomato plants infected with rosette virus failed to develop the characteristic shoestring effect. It was suspected that absence of these symptoms might have been

due to the low light intensities that prevailed in the experimental greenhouses at this time. Therefore, two tomato plants in 8-in. pots were inoculated late in December with rosette virus; after infection became apparent, one was placed under a 500-watt lamp which was intermittently operated (10 min. off and 10 min. on) from 6 p. m. to 6 a. m. The other plant was not given extra illumination. By the middle of February, the plant without extra illumination was beginning to show evidence of shoestring and by the end of March the plant as a whole had a witch's-broom appearance due to the replacement of many leaves by tendrils. The plant that was given extra illumination grew much more vigorously than the other. It had mild green mottling symptoms with some curling and distortion of leaves but failed to develop the shoestring effect. From the results, it would appear that low light intensity was not the primary reason for failure of tomato plants to develop shoestring during the winter months; the results actually suggest, on the contrary, that low light intensity induces shoestring. This symptom has, however, developed both in the field and in the greenhouse during bright sunny days of midsummer when temperatures for several weeks rose above 90° F. Thus, while low temperature favors production of fern-leaf and shoestring symptoms in tomato plants infected with the ordinary strain of tobacco mosaic virus (2, 8), it does not seem to be the primary auxiliary factor in the case of rosette.

When transmitted to *Nicotiana glutinosa* L. and to Holmes' necrotic type tobacco, a derivative from *N. digluta* Goodspeed & Clausen × *N. tabacum* L., the rosette virus produced numerous necrotic local lesions similar to those caused by the type strain of tobacco mosaic virus. The numbers of lesions that developed in these hosts indicate that the virus reached a moderately high concentration in tobacco and tomato plants. Single-lesion strains have been isolated by subinoculation from lesions produced in *N. glutinosa*. Such isolates produced shoestring symptoms in tomato plants to which they were transferred. This is considered evidence that the disease found in the field is due not to infection with a virus complex but to a single virus strain.

In Turkish tobacco plants, the rosette virus produced, on inoculated leaves, numerous yellow lesions which became evident 6 or more days after inoculation. By this time the virus had become systemic in the tobacco plants where it caused veinclearing followed by mild mottling (Fig. 2, A). Under some conditions, yellow rings 3 mm. to 1 cm. in diameter developed on systemically affected leaves; in other cases, however, such leaves developed only mild mottling. The disease in Turkish tobacco proved extremely mild in contrast to the severe disease in tomato. It caused little or no malformation of leaves and relatively little stunting.

Rosette virus failed to produce necrotic local lesions in the Early Golden Cluster variety of garden bean (*Phaseolus vulgaris* L.) under conditions that permitted production of large numbers of such lesions on comparable plants inoculated with other strains of tobacco mosaic virus. In failure to infect this variety of bean, the rosette virus is similar to the ribgrass strain

of tobacco mosaic virus (4), although it differs from it in many other respects.

Rosette virus produced necrotic local lesions, and failed to become systemic, in *Nicotiana sylvestris* Spegaz. & Comes. In this respect it resembles the tomato aucuba strains, the ribgrass strain, and the tomato shriveling strain of tobacco mosaic virus (3, 4, 6).

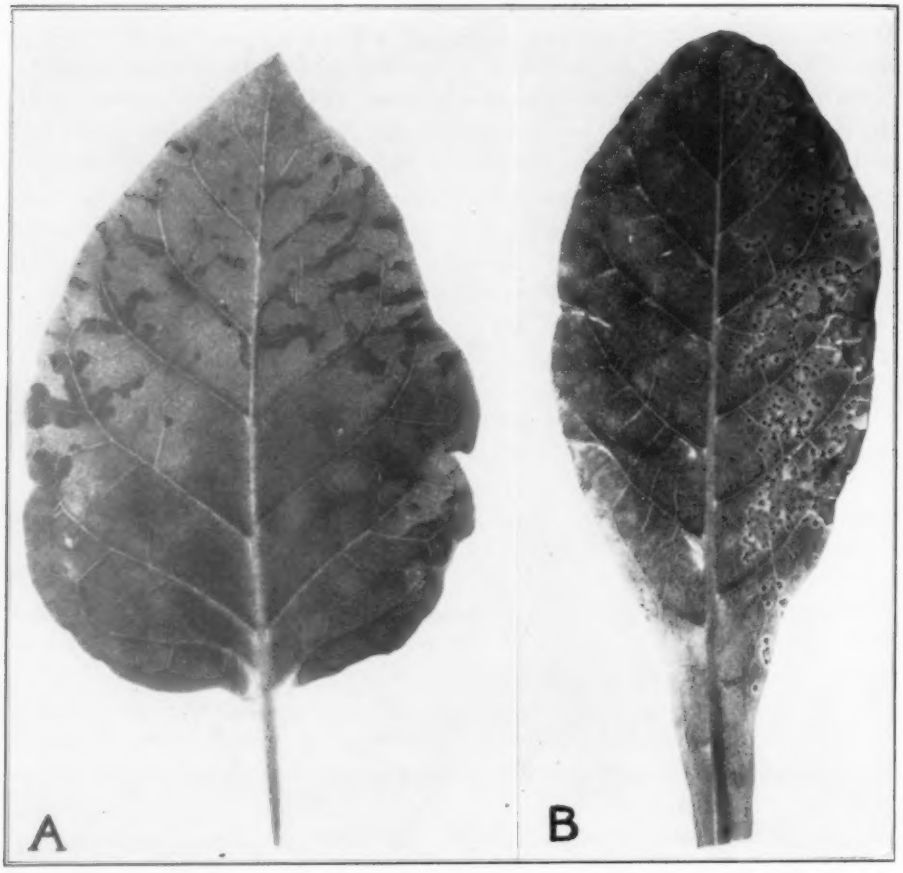

FIG. 2. A. Leaf of an experimentally infected tobacco plant showing green mottling symptoms produced by tomato rosette virus. B. Leaf from a plant of *Nicotiana sylvestris* inoculated first on the left half with the type strain of tobacco mosaic virus, and 4 days later on both halves with tomato rosette virus. Necrotic lesions have developed only on the unprotected half.

IDENTIFICATION OF THE CAUSAL AGENT

The facts that tomato rosette virus produced necrotic local lesions in *N. glutinosa* and necrotic-type tobacco and that it caused mottling symptoms in Turkish tobacco resembling those caused by tobacco mosaic virus suggested a relationship of the rosette virus to the latter. A number of tests were carried out to determine whether or not such a relationship could be demonstrated.

Intracellular inclusions. Leaves from tobacco and tomato plants in-

fected for 3 weeks or longer with the rosette virus were examined under the microscope for the presence of intracellular inclusions. Crystalline plates similar to those present in plants infected with strains of tobacco mosaic virus (5, 9) were abundant in trichomes and epidermal cells of infected tobacco leaves. They were also found in infected tomato leaves but not so abundantly. Association of these plates with the rosette virus suggests its relationship to tobacco mosaic virus.

Thermal inactivation. In two separate tests, 1-ml. samples of freshly expressed sap from tobacco plants infected with the rosette virus were placed in test tubes and heated for 10 min. at 80°, 85°, 90°, and 95° C. in a thermostatically controlled water bath. Infectivity remaining in each of the heated samples was determined by inoculation of five leaves of a single plant of *Nicotiana glutinosa* L. The data obtained are summarized in table 1. The thermal inactivation point of the rosette virus is of the same

TABLE 1.—*Number of lesions produced on five leaves of N. glutinosa inoculated with samples of tomato rosette virus heated to various temperatures for 10 min.*

Temperature (° C.)	Test 1	Test 2
Unheated control	7520	10600
80	3595	2370
85	2030	209
90	1008	4
95	175	0

order of magnitude as that of the type strain of tobacco mosaic virus (7), being between 90° and 95° C. The apparent survival of virus in test 1 at 95° C. for 10 min. may or may not be erroneous. Further tests will be required to determine the validity of this test.

Cross protection tests. The right half of each of five leaves on each of two plants of *N. sylvestris* was rubbed thoroughly with the type strain of tobacco mosaic virus. Four days later, both halves of the ten leaves were rubbed with sap containing rosette virus. Local lesions appeared in large numbers on the left halves of the ten leaves in 3 days, but in much smaller numbers or not at all on the right halves of the same leaves even after 5 days or longer (Fig. 2, B). Results of the test show that the type strain of tobacco mosaic virus protects plants of *N. sylvestris* from infection with tomato rosette virus and thus bring evidence that tomato rosette virus is immunologically related to tobacco mosaic virus.

Complement fixation test. Antiserum prepared in rabbits by injection with a purified preparation of the type strain of tobacco mosaic virus was used in this test. The experimental procedure was essentially the same as that described by Tall, Price, and Wertman (11), the usual controls being included. Crude sap from a Turkish tobacco plant inoculated 3 weeks previously with tomato rosette virus was used, in various dilutions, as antigen. Results of the test are in table 2.

Tomato rosette virus reacted with tobacco mosaic virus antiserum, the serum dilution end-point being taken as 1 : 80 and the antigen dilution end-point as 1 : 800. The serum dilution end-point determined with a purified preparation of the type strain of tobacco mosaic virus as antigen in a control test carried out at the same time was 1 : 640. The data demonstrate a serological relationship between tomato rosette and tobacco mosaic viruses.

TABLE 2.—*Results of complement fixation test with tomato rosette virus in crude sap as antigen and tobacco mosaic virus antiserum*[a]

Antiserum dilution	Antigen (rosette virus) dilution								Serum control
	1: 100	1: 200	1: 400	1: 800	1: 1600	1: 3200	1: 6400	1: 12800	
1: 40	4	4	4	4	4	4	4	1	0
1: 80	4	4	4	4	3	3	2	1	0
1: 160	2	2	2	3	2	+	1	0	0
1: 320	0	+	0	0	0	0	0	0	0
1: 640	0	0	0	0	0	0	0	0	0
1: 1280	0	0	0	0	0	0	0	0	0

[a] Strength of the antigen-antiserum reaction is indicated as follows: 0, complete hemolysis (no reaction); +, 90–95 per cent hemolysis; 1, 75 per cent; 2, 50 per cent; 3, 25 per cent; and 4, none.

The symptomatology and properties of the causal agent, together with results of cross immunological and serological tests, demonstrate that tomato rosette is caused by a strain of tobacco mosaic virus believed to be distinct from those earlier recognized. A technical description of the causal agent follows.

Marmor tabaci H. var. **rosettae** n. var.

Common name: Tomato rosette virus or tomato rosette strain of tobacco mosaic virus. Differs from the type strain in symptomatology on tobacco, tomato, and *Nicotiana sylvestris*, in lack of infectiousness for the Early Golden Cluster variety of garden bean, and serologically.

Hosts: SOLANACEAE—*Lycopersicon esculentum* Mill., tomato; *Nicotiana tabacum* L., tobacco; *N. glutinosa* L.; *N. sylvestris* Spegaz. & Comes.

Nonsusceptible species: *Phaseolus vulgaris* L., bean, var. Early Golden Cluster.

Geographical distribution: Virginia, U. S. A.

Induced disease: In tomato, mild green mottling, severe distortion leading to shoestring, rosette, and witch's-broom effects. In tobacco, mild green mottling frequently accompanied by formation of yellow zonate ring spots. In *Nicotiana sylvestris*, necrotic local lesions.

Transmission: By inoculation of expressed juice in dilutions up to 1 : 1000 or higher.

Thermal inactivation: Between 90° and 95° C. in 10 min.

DISCUSSION

So far as can be determined by reference to the literature, tomato rosette is caused by a distinct, and apparently new, strain of tobacco mosaic virus.

The strain it resembles most closely is *Marmor tabaci* H. var. *deformans* H., which causes tomato enation mosaic (1, 10). The enation mosaic virus, like the rosette strain, produces shoestring and fern-leaf symptoms in tomato. However, it causes enations on the underside of affected tomato leaves, a symptom that the rosette strain has not been observed to induce. Rosette is also distinct from the shriveling disease of tomato which Doolittle and Beecher (3) found to be caused by *M. tabaci* H. var. *siccans* D. & B. The shriveling strain, in common with the rosette and aucuba strains, causes necrotic local lesions in *Nicotiana sylvestris*. Such lesions are of great diagnostic value in identifying strains of tobacco mosaic virus.

The shoestring symptom of rosette closely resembles that produced in tomato by ordinary cucumber mosaic virus (2, 8). The witch's-broom symptom pictured by Doolittle (2) could easily be mistaken for that of rosette. Differentiation can, however, be readily made by tests on *N. glutinosa* or other host plants.

Tomato rosette occurring in field-grown plants may be confused with 2,4-D injury. Unfortunately, many home gardeners use a garden sprayer as a receptacle for 2,4-D to control weeds in lawns and subsequently use the same sprayer, after vain attempts to remove 2,4-D residues, for spraying tomato plants. This poses a problem in identification for phytopathologists in regions where tomato rosette occurs. The problem can be solved if it is remembered that rosette is transmissible from plant to plant whereas 2,4-D injury is not and that plants recover from 2,4-D injury but not from rosette.

SUMMARY

An infectious, leaf-deforming, yield-reducing virus disease of tomato called tomato rosette was observed in various sections of Virginia in 1949 and 1950. The disease is characterized by severe stunting resulting from fern-leaf, rosette, shoestring, or witch's-broom symptoms. The causal virus is readily transmitted by mechanical inoculation (rubbing) in dilutions up to 1 : 1000 from tomato to tomato, tobacco, *Nicotiana glutinosa,* and *N. sylvestris* where it causes symptoms similar to those caused by strains of tobacco mosaic virus, but not to the Early Golden Cluster variety of garden bean. It produced crystalline plates in cells of tobacco and tomato plants, is inactivated by exposure for 10 min. to 90°–95° C., and is immunologically and serologically related to tobacco mosaic virus. It was given the trinomial *Marmor tabaci* H. var. *rosettae* n. var.

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LITERATURE CITED

1. AINSWORTH, G. C. "Enation mosaic" of tomato caused by a virus of the tobacco virus 1 type. Ann. Appl. Biol. **24**: 545–556. 1937.
2. DOOLITTLE, S. P. Tomato diseases. U. S. Dept. Agr. Farmers' Bul. 1934. 1943.
3. ————————, and F. S. BEECHER. A strain of tobacco-mosaic virus causing a necrosis and shriveling of tomato foliage. Phytopath. **32**: 986–994. 1942.
4. HOLMES, F. O. A distinctive strain of tobacco-mosaic virus from Plantago. Phytopath. **31**: 1089–1098. 1941.
5. IWANOWSKI, D. Über die Mosaikkrankheit der Tabakspflanze. Zeitschr. f. Pflanzenkr. **13**: 1–41. 1903.
6. KUNKEL, L. O. Local lesions in Aucuba mosaic of tomato. (Abstr.) Phytopath. **22**: 16. 1932.
7. PRICE, W. C. The thermal death rate of tobacco-mosaic virus. Phytopath. **23**: 749–769. 1933.
8. SEVERIN, H. H. P. Multiple viruses of tomato inducing fruit malformation and leaf symptoms. Hilgardia **20**: 109–136. 1950.
9. SHEFFIELD, F. M. L. The formation of intracellular inclusions in solanaceous hosts infected with aucuba mosaic of tomato. Ann. Appl. Biol. **18**: 471–493. 1931.
10. SMITH, KENNETH M. New virus diseases of the tomato. Jour. Roy. Hort. Soc. **60**: 448–451. 1935.
11. TALL, M. G., W. C. PRICE, and KENNETH WERTMAN. Differentiation of tobacco and tomato ringspot viruses by cross immunization and complement fixation. Phytopath. **39**: 288–299. 1949.

STUDIES ON THE PHYSIOLOGY OF SCLEROTINIA SCLEROTIORUM[1]

SELAHATTIN TANRIKUT AND EDWARD K. VAUGHAN[2]

(Accepted for publication August 16, 1951)

The literature concerning *Sclerotinia sclerotiorum* (Lib.) DBy. contains many references to the etiology and control of white mold and to descriptions of the disease on its many host plants. Very little has been written concerning the physiology of the fungus. These investigations were undertaken in the hope that they might help to clarify certain aspects of the physiology of the fungus and aid in understanding the complex factors that influence its spread and development in the field. Indirectly, such information as is presented may indicate lines of investigation which will result in the development of better control measures.

TEMPERATURE

The influence of temperature on the growth of the fungus and upon infection and development of the mycelium within the host has been noted by Lauritzen, Harter, and Whitney[3] and Ramsey,[4] who obtained infection of snap beans at temperatures ranging from 0° to 28° C. Infection occurred very slowly at 0° to 2° C. The maximum number of infections occurred at temperatures ranging from 19° to 24° C.

To determine the effect of temperature on the growth *in vitro* of *S. sclerotiorum*, five isolates from various parts of the United States were grown on potato-dextrose agar at temperatures ranging from 0° to 30° C. Little growth occurred at 0° C. The rate increased with each increase in temperature up to 20° C., at which most rapid growth occurred. Above 20° C. the rate of growth decreased, although all cultures grew fairly well at 30° C.

Cultures of *Sclerotinia* grown at room temperature tend to produce the same size of sclerotia for a given species, provided a suitable medium is used. In culture, however, Ramsey[4] noted that the relative size of sclerotia does not remain the same when extremes of temperature are involved. In general, when the temperature is near the minimum for the fungus the sclerotia have a tendency to be larger than those produced on the same medium at higher temperatures. Similar results were obtained in these tests. At 5° C. the sclerotia were almost twice as large as those produced at 20° C.

[1] Published as Technical Paper No. 686, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Botany and Plant Pathology. A resume of a thesis presented by the senior author in partial fulfillment of the requirements for the Master of Science degree from Oregon State College.

[2] Plant Pathologist, Oregon Agricultural Experiment Station.

[3] Lauritzen, J. I., L. L. Harter, and W. A. Whitney. Environmental factors in relation to snap bean diseases in shipment. Phytopath. 23: 411–445. 1933.

[4] Ramsey, G. B. Sclerotinia species causing decay of vegetables under transit and market conditions. Jour. Agr. Res. [U.S.] 3: 597–632. 1925.

HYDROGEN-ION CONCENTRATION

Several tests were made to determine the effect of hydrogen-ion concentration on the rate and character of growth of *S. sclerotiorum* in culture. H-ion concentration was determined with the Beckman pH meter, using a calomel electrode. HCl and NaOH were added to the agar after sterilization to adjust the reaction to the desired level, and no attempt was made to maintain the pH of the media after the plates were seeded.

At pH 2.0 all isolates made some growth but there were great differences in the rate and character of growth; all made fair growth at pH 2.4. From pH 3.0 to 8.9 inclusive, all cultures made rapid growth and there were no significant differences in the rate or character of growth of the various isolates. At pH 9.6 the rate of growth of all cultures was retarded and at pH 10.2 very slight growth occurred. No growth occurred at pH 10.5 or above. Thus the limits within which *S. sclerotiorum* can grow appear to be approximately pH 2 to pH 10.

MINERAL NUTRITION

The amount of Mg, Fe, S, and K in the substrate had no apparent effect on the rate or character of growth of *S. sclerotiorum*. Five isolates grew equally well when these elements were absent, when they were present in usual amounts, and when excessive quantities were present. Of the nutrients tested only nitrogen, phosphorus, and carbohydrates appeared to be of any importance in the growth and formation of sclerotia.

Inorganic nitrogen appeared to have a greater effect on growth of *S. sclerotiorum* than any other single element. When no nitrogen was present the fungus covered the surface of the agar plates in 3–5 days and formed sclerotia in 7–9 days. Growth, however, was weak, thin, and composed almost entirely of sessile mycelia, and few sclerotia were formed. When nitrogen was present a felt of aerial mycelia was formed and numerous sclerotia were present. Most rapid growth occurred on agar containing 1–5 gm. of $NaNO_3$ per liter. Higher concentrations depressed the rate of radial growth but did not interfere with the formation of aerial mycelium or sclerotia.

Most isolates grew well when ammonia nitrogen was substituted for nitrate. On a combination of nitrate and ammonia nitrogen (NH_4NO_3) all isolates grew luxuriantly and formed numerous sclerotia, but growth was not better or worse than when either form was used alone.

Three organic forms of nitrogen had quite different effects on the growth of *S. sclerotiorum*. When cystine ($C_6H_{12}O_4N_2S_2$) was used as the source of nitrogen, growth was similar to that obtained when no nitrogen was present. On the other hand, on media containing asparagine ($C_4H_8N_2O_3$) as the source of nitrogen, growth was luxuriant and the sclerotia formed were larger than those formed on any other medium.

Urea (NH_2CONH_2) was toxic to the fungus at concentrations greater

than 0.5 gm. per liter. Rate of growth was retarded appreciably when only 1 gm. of urea was used per liter, and no growth at all occurred when more than 3.5 gm. was used. Whenever any growth occurred on media containing urea, all cultures eventually covered the surface of the agar plates and formed sclerotia. These latter were much larger than those formed on ordinary culture media and comparable with those formed on media containing asparagine.

A lack of phosphorus was evidenced not by a reduction in rate of radial growth but by the production of a very thin, sparse mycelium. Growth was most rapid and most luxuriant on media containing 5–10 gm. NaH_2PO_4 per liter. More than 10 gm. per liter caused a reduction in rate of growth and formation of sclerotia.

The type of carbohydrate used appeared to have little effect on the rate of radial growth, but carbohydrates were essential for the formation of sclerotia. Growth was equally good when dextrose, sucrose, raffinose, inulin, and potato starch were used. Best mycelial growth occurred when 25–50 gm. per liter were used, but more sclerotia were formed at 25 gm. per liter than at either lesser or greater concentrations.

PHYSIOLOGIC SPECIALIZATION

Ramsey[5] made numerous cross-inoculation tests to determine whether there were physiologic races of *S. sclerotiorum* and other closely related species. His results indicated that the host range of *Sclerotinia* spp. is so wide that it was impossible to distinguish races on the basis of host infection. In the course of these investigations some evidences of specialization were obtained, based entirely on physical reactions of the isolates to differences in hydrogen-ion concentration and temperature.

At pH 2.0 isolate A (from Corvallis, Oregon) consistently grew well, producing a thin felt of mycelium with numerous sclerotia (Fig. 1). Isolate 27C (from Mt. Vernon, Washington) produced very dense colonies more than 2 mm. thick but only 2 cm. in diameter after 13 days. Isolate F (from Fort Lauderdale, Florida) was not so uniform nor so consistent as the other two, but occupied a position approximately midway between these two extremes.

At low temperatures all isolates grew equally well and there were no apparent differences between them. On the other hand they could be separated readily into two distinct groups when grown at high temperatures. The rate of growth of some isolates (27B, 27C, and 34) increased rapidly with rising temperature up to 20° C., at which they made most rapid growth. Above that temperature the rate of growth decreased rapidly (Fig. 2). The rate of growth of another group of isolates (27S and 35) also increased rapidly up to 20° C. These isolates, however, grew well at temperatures as high as 30° C.

[5] See footnote 4.

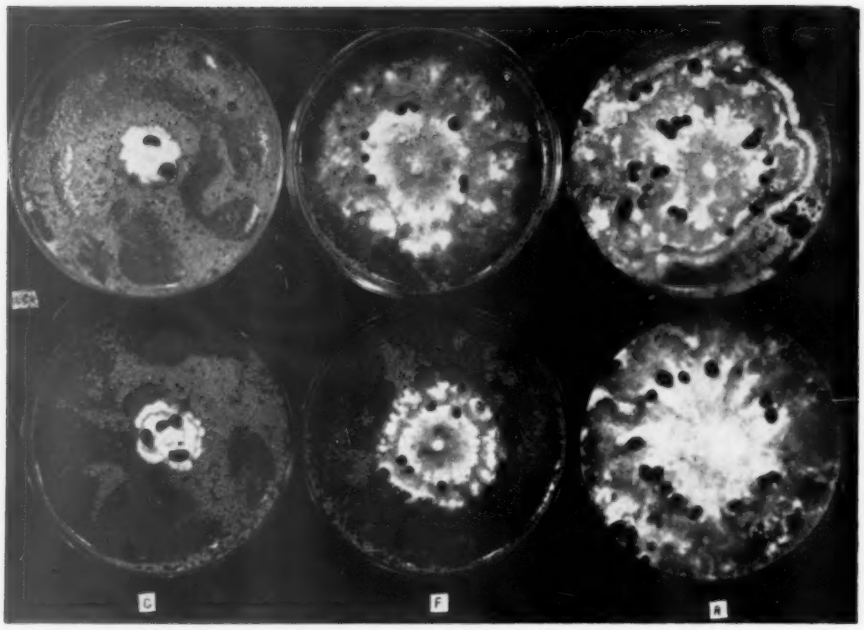

FIG. 1. Growth of three isolates of *Sclerotinia sclerotiorum* on Richards' solution agar at pH 2.0.

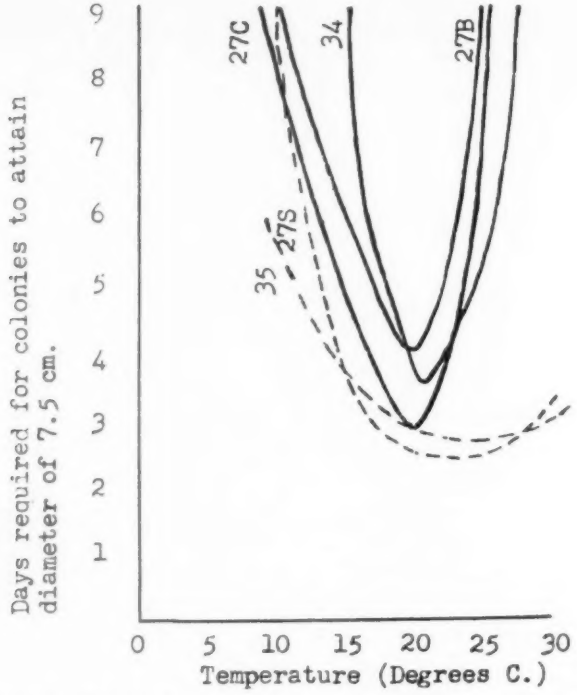

FIG. 2. Growth of five cultures of *Sclerotinia sclerotiorum* on potato-dextrose agar at temperatures ranging from 5° to 30° C.

DISCUSSION

Sclerotinia sclerotiorum attacks a wide variety of economic crops and weeds, and under certain conditions is able to live for long periods as a saprophyte. These facts indicate that its nutrient requirements are not specific. These investigations have repeatedly shown that this is true. While the fungus did show preferences for certain elements, it also had a bewildering ability to grow on almost any substrate, regardless of the presence or absence of any specific mineral or organic nutrient. Such freedom from rigid nutrient requirements makes it an almost ideal parasite.

Although all cultures grew best at 15° to 25° C., they were able to grow and produce sclerotia in a range from 0° to 30° C. Thus the fungus can at least maintain itself at almost any temperature that is suitable for the growth of crop plants. In addition it can grow quite well on media of pH 2.4 to 9.6, a tremendous range from extremely acid to very alkaline.

This extreme adaptability, plus its habit of producing tough, resistant sclerotia which withstand unfavorable periods, make it an unusually difficult fungus to control. The most promising factor indicated by these investigations is its sensitivity to certain chemicals such as urea. The use of such materials for its control is already under investigation.

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THE PRODUCTION OF A TOXIC MATERIAL BY ALTERNARIA SOLANI AND ITS RELATION TO THE EARLY BLIGHT DISEASE OF TOMATO[1]

GLENN S. POUND AND MARK A. STAHMANN

(Accepted for publication September 5, 1951)

In previous greenhouse studies (6) of the early blight disease of tomato (*Lycopersicon esculentum* L.) caused by *Alternaria solani* (E. & M.) Jones & Grout, it was observed that chlorotic areas around necrotic lesions were usually more extensive if lesions were located on veins, and that single small lesions located on a leaf petiole might cause the entire leaf to abscise. In other observations it was noticed that when lesions were localized on one side of a petiole, leaflets on the affected side were often chlorotic or necrotic, the other side remaining normal. Experiments were performed to ascertain whether *A. solani* produced a substance toxic to tomato plants and, if so, to what extent such a substance increased defoliation. This paper presents the results of these experiments, which demonstrated the toxin[2] production and illustrated the possible role the material plays in defoliation and in the symptom syndrome of the disease. A preliminary report has already been published (7).

MATERIALS AND METHODS

Bonny Best tomato plants were grown in 4-in. clay pots in greenhouse compost. The isolates of *A. solani* used were, for the most part, those recently described under the same symbol designations (6). The organisms were grown on a modified Czapek medium[3] until they had passed the point of maximum growth, usually 15–20 days. The fermented liquid was decanted off and filtered with a Büchner funnel-filter-flask apparatus, using Whatman No. 42 filter paper or a layer of asbestos and Hyflo Super-Cel. The undiluted filtrate was usually divided into equal portions, one portion left untreated and the other autoclaved for 15 min. at 15 lb. pressure. Tests of activity were then made of the raw and sterilized filtrates.

In testing the filtrates for toxic activity several methods were used. Cuttings of tomato plants were made under water and the cut end immediately immersed in the filtrate. Highly active filtrates produced epinasty immediately and wilting within a few hours. Another very satisfactory test was to clip the end of a petiole of a lower leaf of a tomato plant and insert it in a vial containing the filtrate to be tested. Within a few hours

[1] Published with approval of the Director of the Wisconsin Agricultural Experiment Station.

Acknowledgment is made to Eugene Herrling for making the photographs used in this paper.

[2] Use of the word toxin in this manuscript refers to a metabolic product of *A. solani* which produces disease symptoms when introduced into tomato plants.

[3] The medium consisted of 2 gm. $NaNO_3$; 0.5 gm. KCl; 0.5 gm. $MgSO_4$; 1 gm. K_2HPO_4; 0.01 gm. $FeSO_4$; 30 gm. dextrose; and distilled water to make 1 liter.

wilting and necrosis developed on leaves above the one through which the toxic substance was introduced.

The degree of toxic effect usually shown by wilting and necrosis was recorded as 0, 25, 50, 75, or 100 indicating, respectively, no reaction, slight, moderate, or severe reaction, or death. Intermediate values, such as 15 and 35, were occasionally used when symptoms seemed to require it.

In all tests, sterile unfermented Czapek solution and distilled water were used for controls. In dilution tests with filtrates, distilled water was the diluent.

EXPERIMENTAL RESULTS

Production of a toxic substance in fungus lesions on tomato plants. Tomato plants in a 6- or 8-leaf stage were inoculated by placing drops containing spores or macerated mycelium 1) over the intercostal leaf tissue;

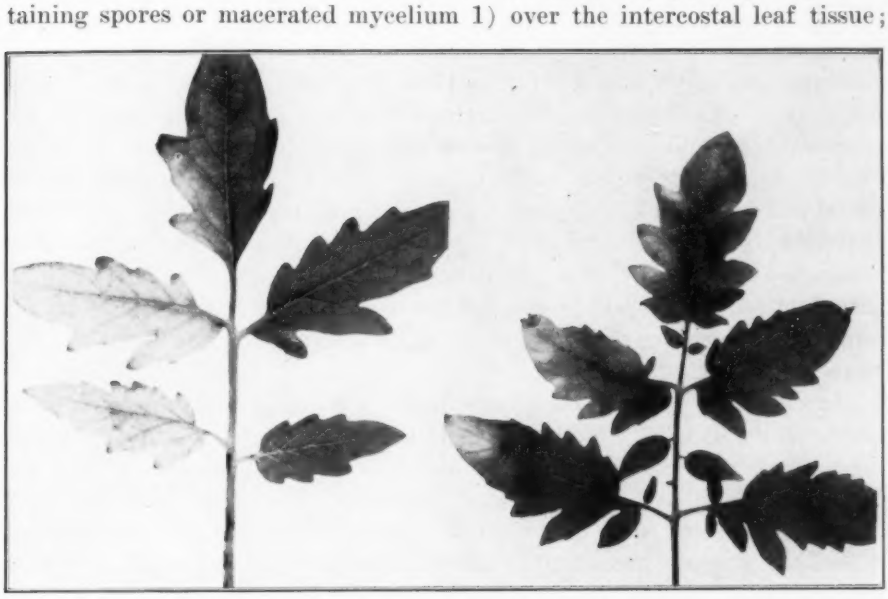

FIG. 1. Chlorosis produced by the toxic principle of *Alternaria solani*. Left, fungus lesion localized on left side of petiole. Note unilateral chlorosis. Right, similar unilateral chlorosis produced by introducing a sterile dilute filtrate of *A. solani* through a lower petiole.

2) at the base of the petiole on one side or 3) all around the base of the petiole; 4) on main stem toward the middle of the plant; or 5) on main stem at the soil surface to produce the collar-rot phase of the disease.

All isolates gave similar results. When lesions were localized on one side of the petiole, two types of reaction occurred after several days' incubation. With the first type, leaflets on the side affected and distal to the lesion became chlorotic. Ultimately, the leaf was conspicuously and completely unilaterally chlorotic, the unaffected side remaining dark green (Fig. 1). When the petiole was completely girdled at the base, the entire leaf became chlorotic. The fungus could not be isolated from tissue outside the lesion

area but was readily isolated from the lesion itself. In the second type of reaction, leaves became severely necrotic instead of chlorotic, again unilaterally, with the necrosis on the side of the lesion (Fig. 2). Efforts to isolate an organism from the affected tissue outside the lesion area itself were unsuccessful.

To ascertain whether the leaflet chlorosis and necrosis resulted from severed vascular elements due to the depth of the fungus lesion, leaves were supported by splints and sections of the petiole were removed, extending to three-fourths the diameter of the petiole. After 30 days of subsequent growth no wilting or chlorosis had developed and the leaves remained turgid and dark green.

Lesions on nonvascular leaf tissue developed less extensively than those on vascular tissue, and little defoliation resulted except when lesions covered the leaf area or when the plant was growing at low temperature (6). One lesion on a petiole of a leaf or leaflet was generally far more effective in producing chlorosis and defoliation than were many lesions on intercostal leaf tissue. Since the toxic material often caused necrosis, perhaps the more extensive necrosis of a vascular lesion was in part produced by the toxin. As the material is evidently transported in vascular tissue, intercostal lesions would be expected to be smaller than vascular lesions. Some inoculations were made by spraying with a water suspension of spores or macerated mycelium. This was done in such a way that leaflets received inoculum while the petiole did not, or vice versa. Disease development and defoliation were decidedly more rapid and more severe when petioles were inoculated.

Inoculations in which the fungus lesion was localized on the main stem, either at the soil level (collar rot) or in the central portion of the plant, often resulted in unilateral wilting and necrosis of upper leaves (Fig. 2). Not all leaves above the inoculated area were affected unless stems were completely girdled with deep lesions. Efforts to isolate the fungus from wilted and necrotic areas on the affected leaves were unsuccessful. Plants with collar rot lesions did not always develop foliage symptoms of a toxic effect, although such was common.

Previously it was reported (6) that tomato plants infected with *A. solani* and growing at 28° C. were defoliated only with vascular infections. Intercostal lesions did not effect defoliation at 28° C. with any isolate studied. Temperature apparently inhibited development of the intercostal lesions at 28°, whereas with the vascular lesions the toxic material was readily transported and caused abscission *per se*. Vascular infections at 28° resulted in extensive necrosis, part of which may have been produced by the toxic substance.

Production of the toxic material by the fungus in culture. When any of the four isolates of *A. solani* studied was grown in Czapek medium for 2 to 4 weeks, a highly toxic substance was found in the fungus filtrate. Autoclaving the filtrate for 15 min. at 15 lb. steam pressure did not affect

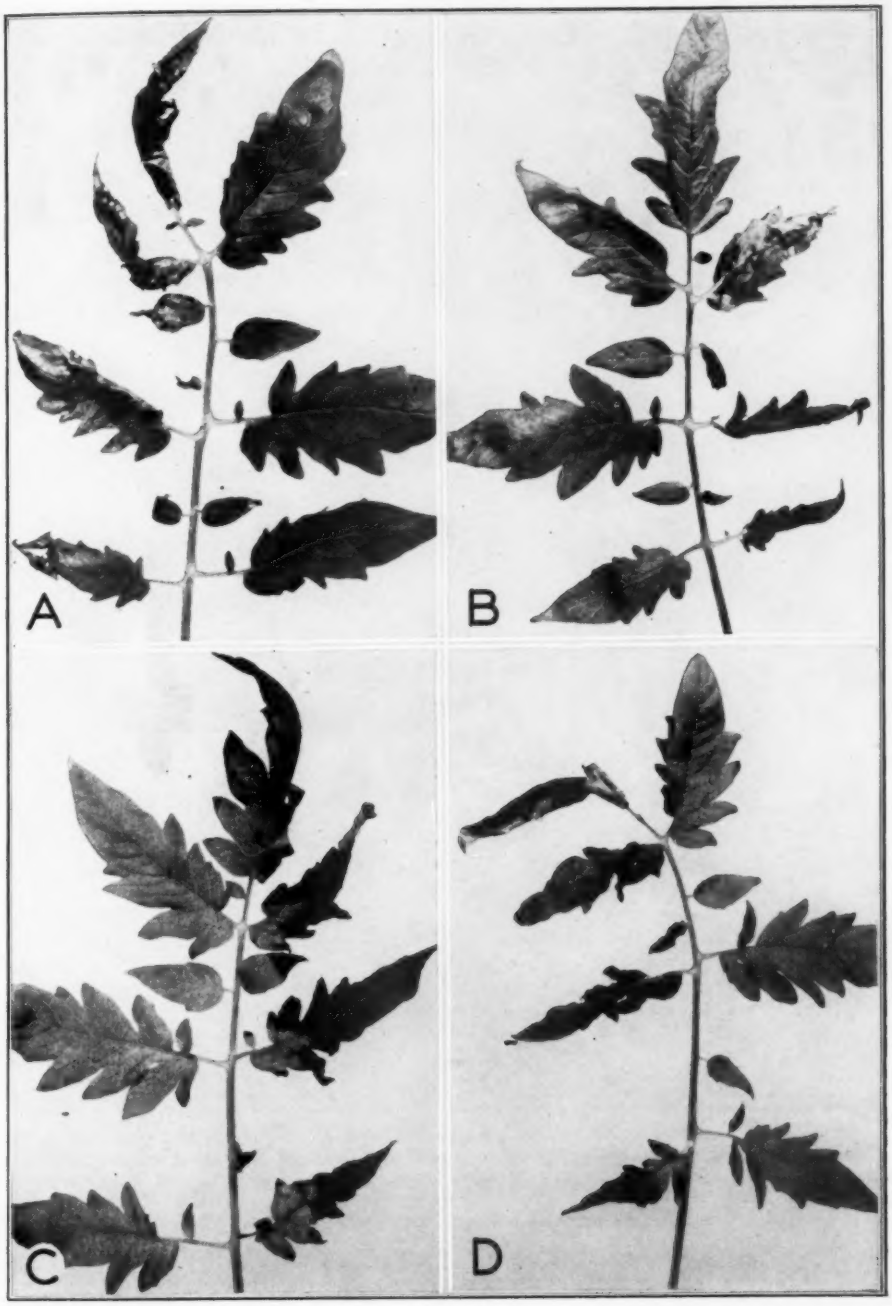

FIG. 2. Unilateral wilting and necrosis produced on leaves of tomato plants when lesion of *A. solani* was localized (A) on the left side of the basal portion of the petiole, or (B) at the ground level of the main stem. Note identical symptoms produced by introducing (C) a sterile filtrate of *A. solani* into a lower petiole, and (D) crystalline toxin at a dilution of 1: 250,000 into a lower petiole.

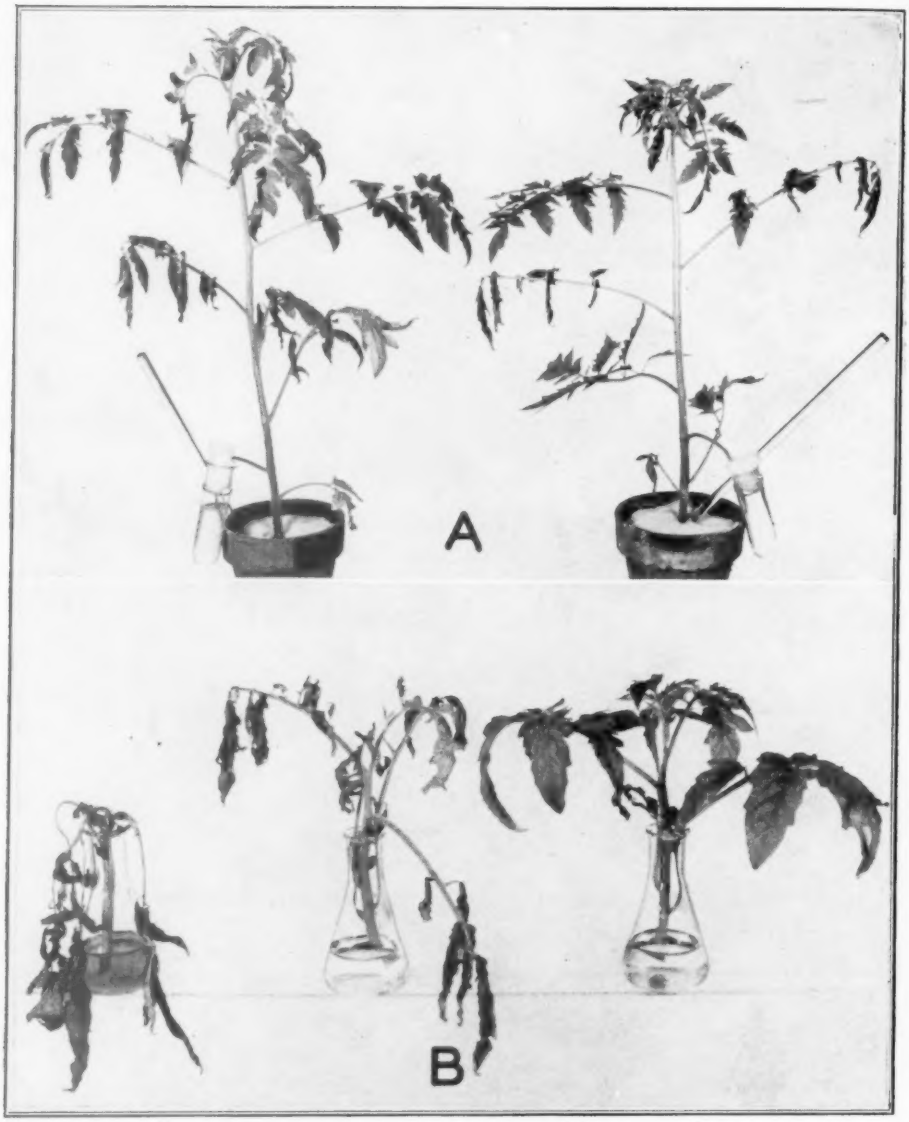

FIG. 3. A, unilateral wilting and necrosis of tomato leaves produced by introducing a sterile filtrate of *A. solani* through lower petiole (left), and a solution (1: 250,000) of the crystalline toxin (right). B, wilt and necrosis of tomato cuttings 30 hr. after they were placed in a sterile filtrate of *A. solani*. Left, filtrate undiluted; center, filtrate diluted 1: 25; right, Czapek medium control.

the toxic activity. When tomato cuttings were placed in the filtrates rapid wilting and necrosis followed (Fig. 3). Leaves often developed circular necrotic lesions and necrotic cankers developed on the stem, symptoms which resembled closely the field symptoms of early blight (Fig. 4).

When the filtrate or a solution of the crystalline toxin was introduced into a growing plant by immersion of the cut end of a lower petiole in the

filtrate, unilateral wilting and necrosis of upper leaves and epinasty of the growing point followed (Fig. 3). These symptoms were indistinguishable from symptoms produced by fungus lesions on the plant (Fig. 2).

Often when fungus lesions were localized on one side of a petiole, chlorosis rather than necrosis was produced on leaflets distal to the lesion. When cuttings were placed in the fungus filtrates, necrosis and wilting usually followed. The necrotic reaction to undiluted filtrates was very rapid. However, if the filtrate was diluted sufficiently and introduced

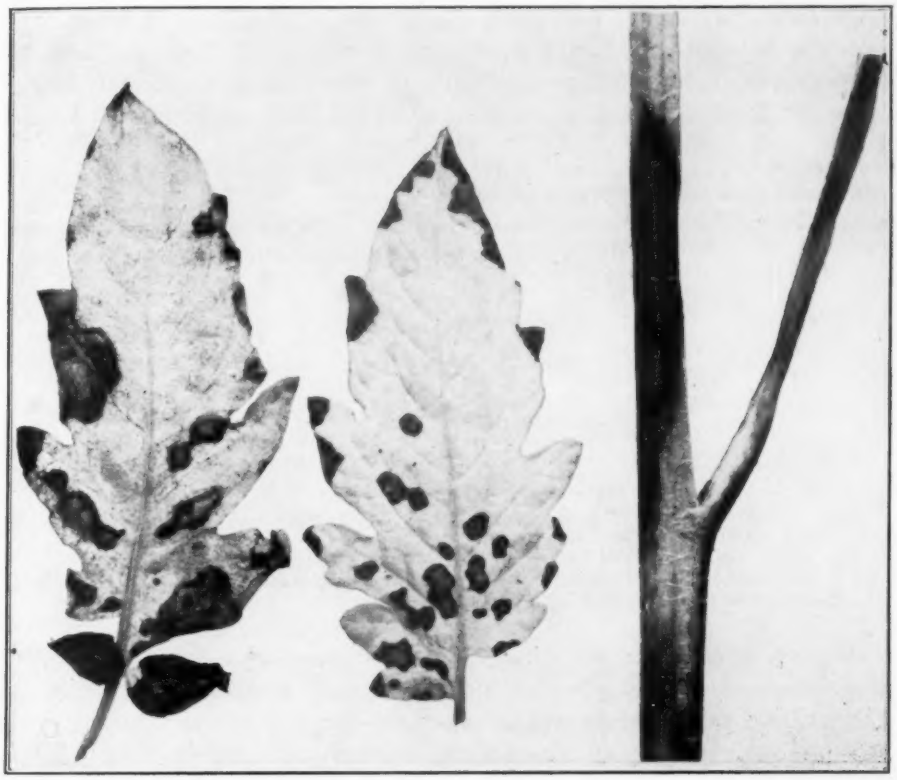

FIG. 4. Stem canker and circular necrotic lesions on leaf of tomato cutting 48 hr. after it was placed in filtrate of *A. solani*. A solution of the crystalline toxin produced very similar effects.

through a petiole into a growing plant, unilateral leaf chlorosis identical to that produced by fungus lesions was obtained after several days (Fig. 1).

Stem vascular anatomy was indicated by leaf symptom development. When a toxic filtrate was introduced into a lower leaf petiole, the reaction on affected individual leaves above was unilateral and only part of the leaves were affected. When the toxic principle was introduced simultaneously through two lower petioles, practically all the leaves above were affected and plants were often killed. The toxic agent was apparently not translocated from one plant organ to another but was kept within the vas-

cular trace into which it was introduced, and the symptoms indicated that the vascular tissue of the entire petiole receiving the solution was connected to only one side of many of the leaves above.

The toxic material was readily taken in through the roots of young seedlings growing in sand culture but it caused only a slight reaction when sprayed on foliage.

Relative production of the toxic material by different isolates of Alternaria solani. To test the relative activity of the toxic materials produced by the various isolates of *A. solani,* each of the isolates was grown in aliquots of one batch of medium. After 7 weeks' growth, each filtrate was tested with tomato cuttings in a series of dilutions. The results (Table 1) show that the toxic substance produced by isolate C was moderately active when diluted 1000 times. In several trials the toxic agent of this isolate

TABLE 1.—*Relative activity of the toxic material produced by various isolates of Alternaria solani grown on Czapek medium*

Isolate	Disease index of tomato cuttings at filtrate dilution shown							
	None	1:2	1:4	1:10	1:25	1:50	1:100	1:1000
A	75[a]	75	65	25	15[b]	10[b]	10[b]	0
B	100	85	25	0	0	0	0	0
C	100	100	100	100	100	85	75	50
D	85	65	50	25	10	0	0	0
E	100	100	85	75	65	25	0	0
A. tomato[c]	15	15	0	0	0	0	0	0
Czapek control	10[d]	0	0	0	0	0	0	0

[a] Values are averages of four replications and were taken after 96 hr.
[b] Only slight wilting and chlorosis.
[c] Culture kindly supplied by Dr. G. F. Weber.
[d] Unfermented Czapek medium often caused very slight marginal burning of leaflets, especially at low temperature.

was much more active than that of other isolates, although all produced similar symptoms. Isolate D, which was almost identical to isolate C in culture and pathogenicity, consistently produced a less active substance. Isolate E, a pigment-producing isolate, in several tests appeared to be identical in culture and pathogenicity to isolate A. It was isolated from the same infected leaf which yielded isolate C. It is interesting that *Alternaria tomato* Weber (9), which produces a disease on foliage very similar to that caused by *A. solani,* gave little evidence of production of a toxic substance in these trials.

Effect of air temperature on toxic activity. Since isolate A was found in earlier work (6) to produce less disease at 28° C. than isolates C and D, even when vascular tissue was invaded, it was of interest to see whether air temperature affected the response of tomato cuttings to the toxic material of the various isolates. Filtrates of isolates A, C, and D were tested in a series of dilutions at 16°, 20°, 24°, and 28° C. All showed essentially the same response to temperature (Table 2); i.e., necrosis and wilting increased

with increase in temperature. Development was much more rapid at high temperatures, probably because of the effect of transpiration on the rate of solution intake.

Reaction of other plants to the toxic substance. Various species of plants were tested as cuttings in a highly active filtrate of isolate C: pumpkin, cabbage, radish, spinach, sugar beet, tobacco, pea, bean, sweet clover, cowpea, and poinsettia. In all but pumpkin rapid wilting and severe necrosis developed. Pumpkin cuttings eventually had some leaf burning but effects were much less than for other plants.

Isolation and properties of the toxic material. Preliminary experiments on the isolation of the toxic material showed that it could be extracted by alcohol from the residue remaining after concentration of the culture filtrates. While the properties of these highly active toxic concentrates were

TABLE 2.—*Relative activity of the toxic substance to tomato cuttings at various air temperatures and dilutions*

Isolate and temperature (° C.)	Disease index of tomato cuttings after 96 hr. at filtrate dilution shown				
	None	1: 10	1: 50	1: 100	1: 1000
A—16	85	50	15	10	0
20	85	50	15	10	0
24	85	35	15	10	0
28	100	50	15	10	0
C—16	100	100	50	50	0
20	100	100	85	75	0
24	100	100	85	75	25
28	100	100	100	85	50
D—16	75	25	15	10	0
20	75	35	15	10	0
24	85	35	25	10	0
28	85	50	25	10	5

being studied, the paper by Brian et al. (1) appeared, describing a biologically active metabolic product from *Alternaria solani* which they called alternaric acid. Since the properties of this product coincided closely with those of our toxin from the alcoholic extracts, the method for the isolation of alternaric acid described by them was applied to our culture filtrates. Crystals were readily obtained by extracting the acidified culture filtrate of strain C with chloroform and dissolving in carbon tetrachloride the gummy residue left after evaporation of the chloroform. The crystals thus obtained were purified by dissolving them in dilute sodium bicarbonate solution followed by precipitation with dilute hydrochloric acid, and also by recrystallization from benzene or water.

Although the general properties of the crystals which we have obtained seemed to be similar to those reported by Brian et al. (1) for alternaric acid, our preparations generally melted somewhat below the 134° C. melting point they reported. We have obtained crystals melting at 130° to 132° C.; however, this melting point varies somewhat with the rate and time of heat-

ing. Other preparations of the acid generally had somewhat lower melting points. It thus appeared that our isolate C of *A. solani* produced a small amount of closely related substances which were difficult to separate from the alternaric acid.

A sample of crystalline alternaric acid, kindly submitted to us by Dr. P. W. Brian, was compared with the crystalline toxin isolated from our cultures by means of mixed melting point determinations and ultraviolet absorptions, and by observing the effects which each sample produced on tomato plants. The mixed melting point determinations indicated that by this test the two samples were essentially the same. The characteristic ultraviolet absorption spectrum of the alternaric acid supplied by Dr. Brian was identical with that of the crystalline toxin isolated from our cultures. The epinasty, wilting, and necrosis that were produced when tomato cuttings were placed in parallel serial dilutions, or when either preparation was introduced into tomato plants through leaf petioles, developed simultaneously and showed the same characteristic syndrome in the plants receiving the alternaric acid from England and those receiving the crystalline toxin from our cultures.

The crystalline toxin is extremely active in producing chlorosis, leaf lesions, and necrotic streaking of stems—symptoms of plants infected with early blight. It also causes effects indistinguishable from those produced by introducing sterile culture filtrates into tomato cuttings. The crystalline preparations caused epinasty, wilting, and necrosis of tomato cuttings within a few hours after the cut end was immersed in an aqueous solution containing as little as one part of the sodium salt in 50 million parts of water. When the solution was introduced into a growing plant by immersing the cut end of a lower petiole in the solution, the result was typical unilateral wilting and necrosis of upper leaves, together with the epinasty of the growing point previously observed with culture filtrates (Fig. 3). The leaf symptoms so produced by the crystalline toxin at a dilution of 1:250,000 were indistinguishable from those produced by a 1:10 dilution of the culture filtrate (Fig. 2). The crystalline toxin also produced characteristic lesions on the stems, similar to those sometimes observed in infected plants or produced by culture filtrates (Fig. 4). Brian et al. (1) reported that media containing 15 per cent sucrose yielded a much greater concentration of toxin than media containing dextrose. This was also true in these experiments.

DISCUSSION

In 1937 Whipple (10) referred to observations which led him to believe that *Alternaria solani* produced a metabolic substance which produced necrosis on infected tomato plants. Thomas (8) in 1944 reported foliage lesions on plants which were apparently infected only at the soil level. He, too, suggested the possibility of toxin production by the fungus. Recently Brian et al. in England isolated alternaric acid from filtrates of *A. solani* and tested it for antibiotic effects. The crystalline toxin produced by *A.*

solani and described herein is apparently identical with alternaric acid isolated by Brian et al.

Results reported herein indicate that alternaric acid plays an important role in early blight symptoms and in defoliation. Considerable chlorosis and necrosis are a part of the field symptoms of the disease. Alternaric acid produces such symptoms when introduced into the plant. It is felt that if a single fungus lesion is initiated on intercostal leaf tissue it is relatively unimportant except in reproducing the fungus spores. Under very heavy infection, number of lesions on lamina tissue may alone be sufficient to effect defoliation. In contrast, however, if a single fungus lesion is initiated on vascular tissue of leaf or stem from whence the alternaric acid produced can be transported to other plant parts, the chances for defoliation are greatly increased. It has been observed that lesions related to even small veins produce more chlorosis in the surrounding area than lesions which are strictly intercostal. Stem and petiole infections are more important than others in defoliation, especially at high temperatures (6). It is felt that, especially in advanced stages of the disease when defoliation is severe, part of the chlorosis and necrosis seen can be ascribed to alternaric acid rather than to the presence of the fungus. This accounts for certain failures to isolate the organism from necrotic lesions.

Alternaric acid is active at extremely high dilutions, reactions being obtained with tomato cuttings when the acid is diluted as high as 50 million times. Symptoms produced by an aqueous solution of crystalline alternaric acid when introduced into the plant are identical with symptoms produced by crude fungus filtrates.

It is becoming increasingly evident that "toxin" production by many plant pathogens plays an important role in pathogenesis. Thus, certain wilt-inducing Fusaria produce substances which account for the wilting and chlorosis accompanying infection (3, 4, 5, 11). Other examples of the production of toxic metabolites by plant pathogens are to be found in the literature (2, 12). It is probably true that in many defoliation diseases, leaf abscission is increased by toxic substances produced by the pathogen, and other parts of the symptom syndrome may be due to the toxic substances *per se*.

With special reference to early blight of tomato and potato, the difficulty often encountered in securing good fungicidal control of this disease may be explained, in part at least, in the light of these findings. Relatively few lesions located on veins, which result from escape of the fungus from protective chemicals, may cause a rapidly developing systemic toxemia and thus considerable chlorosis and leaf drop may result in spite of effective control judged on the basis of number of lesions per leaflet.

SUMMARY

Alternaria solani (E. & M.) Jones & Grout, the incitant of tomato early blight, has been found to produce a toxic substance which causes chlorosis and necrosis when introduced into tomato plants either from fungus lesions

on the plant or as a fungus filtrate. Unilateral chlorosis and necrosis of leaflets distal to petiole lesions are very common. Also, lesions on the main stem either at the soil level or in the central portion may cause necrosis or chlorosis of leaves distal to the point of infection. When a sterile filtrate of the pathogen was introduced into a lower petiole of a plant, chlorosis and necrosis developed which were indistinguishable from those caused by fungus lesions on the stem. When tomato cuttings were placed in a sterile filtrate of the pathogen, immediate epinasty occurred and wilting and necrosis developed in a few hours. A crystalline toxin, apparently identical with the alternaric acid described by Brian et al., was isolated from the fungus filtrates. This acid produced similar epinasty, wilting, and necrosis at dilutions as great as 1 to 50 million. Isolates of the organism differ in the amount of alternaric acid produced.

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LITERATURE CITED

1. BRIAN, P. W., P. J. CURTIS, H. G. HEMMING, C. H. UNWIN, and JOYCE M. WRIGHT. Alternaric acid, a biologically active metabolic product of the fungus Alternaria solani. Nature **164**: 534–535. 1949.
2. CLAYTON, E. E. Toxin produced by Bacterium tabacum and its relation to host range. Jour. Agr. Res. [U.S.]**48**: 411–426. 1934.
3. FAHMY, T. The production by Fusarium solani of a toxic excretory substance capable of causing wilting in plants. Phytopath. **13**: 543–550. 1923.
4. GÄUMANN, E., and O. JAAG. Über das Problem der Welkekrankheiten bei Pflanzen. [On the problem of wilt disease in plants.] Experientia **2**: 215–220. 1946.
5. GOTTLIEB, DAVID. The presence of a toxin in tomato wilt. Phytopath. **33**: 126–135. 1943.
6. POUND, GLENN S. Effect of air temperature on incidence and development of the early blight disease of tomato. Phytopath. **41**: 127–135. 1951.
7. ————, and M. A. STAHMANN. Production of a toxic substance by Alternaria solani and its relation to the early blight disease of tomato. (Abstr.) Phytopath. **41**: 30. 1951.
8. THOMAS, H. REX. Collar-rot infection on direct-seeded tomatoes. U. S. Dept. Agr., Pl. Dis. Rptr. **24**: 8–10. 1940.
9. WEBER, G. F. Nailhead spot of tomato caused by Alternaria tomato (Cke.), n. comb. Fla. Agr. Exp. Sta. Bul. 332. 1939.
10. WHIPPLE, OTIS C. Macrosporium seedling blight of tomatoes. Wis. Univ. Sums. Doctoral Diss. **3**: 65–66. 1938.
11. WOLF, FREDERICK T., and FREDERICK A. WOLF. A toxic metabolic product of Fusarium oxysporum var. nicotianae in relation to wilting of tobacco plants. Phytopath. **38**: 292–298. 1948.
12. ZENTMYER, G. A. Toxin formation by Ceratostomella ulmi. Science (N.S.) **95**: 512–513. 1942.

NUTRIENT STUDIES WITH THE WHEAT BUNT FUNGUS, TILLETIA CARIES

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In studies of chemical aspects of disease resistance in plants, particularly the resistance of Baart 38 wheat to bunt (3), it was desirable to culture *Tilletia caries* (DC.) Tul. *in vitro*. Preliminary survey of the customary media for growth of this organism revealed that on the best of them (Leonian's and potato-dextrose agar (7)) growth was very slow, at least 8 days being required for visible growth and 2 weeks for an appreciable amount of growth. Several additional days were needed if the cultures were seeded with chlamydospores instead of with transfers from growing cultures. Therefore, nutritional requirements were investigated to develop an improved medium for this fungus. The desirability of reproducible substrates has been stressed recently by Halbsguth (5).

METHODS AND MATERIALS

Bunted kernels from Baart wheat grown in Davis, California, were the source of *Tilletia*. Race T-1 (3, 8) is probably the only race occurring in the sample. Chlamydospores were seeded with a sterile wire loop to various media and, by successive transfers, cultures were produced with no remnants of chlamydospore-wall material.

Chemicals used were of highest purity obtainable from commercial sources, with no attempt made to remove trace impurities. Water was redistilled from alkaline permanganate solution in an all-Pyrex system. Pyrex glassware was used throughout these studies. Later it was found that water from a resinous ion exchange system (Duolite Ion Exchange Apparatus) was equally satisfactory.

Cultures were replicated three to five or more times. Some inequalities among cultures on identical media presumably were due to differences in amount, distribution, or viability of the chlamydospore or transfer material. Most experiments were sufficiently prolonged for such differences to disappear.

An incubation temperature of 19° to 20° C. was employed because growth decreased at 15° and 25° C.

At first such standard media were tried as Leonian's and potato-dextrose agars, Richards' liquid medium (7), and modifications of these with omission or addition of peptone. Growth occurred on Leonian's and potato-dextrose agar but not on Richards'. Sartoris' liquid medium (10) and Barnes' and Blakeslee's agar media (7) supported a slow growth of

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The writer wishes to thank Henry A. Schreiber and Robert B. Griffith for their assistance with parts of these studies.

Tilletia. All were discontinued because they supported very little growth or because they contained at least one mixture, such as malt or potato extract, which could not be chemically defined.

Rates of growth were compared visually except in a few cases. Total amounts of mycelial growth were compared with a control, which represented either a minimal medium (hereafter called MT0) that barely main-

TABLE 1.—*Composition of media*

Concentration of stock solution	Substance	Volume or weight	Resultant composition	
			Molality	Total p.p.m.
Minimal medium (MT0)				
0.2 M	$MgSO_4$	2.5 ml.	0.0005	Mg 12
do	KH_2PO_4	1.5 ml.	0.0003	P 25
do	K_2HPO_4	2.5 ml.	0.0005	K 51
0.5 M	$Ca(NO_3)_2$	1.5 ml.	0.00075	Ca 30
do	NH_4NO_3	0.5 ml.	0.00025	N 28
0.5 per cent	Ferric potassium tartrate	0.5 ml.	.....	.....
	Sucrose	16 gm.	0.047	16000
	H_2O to make 1000 ml.			
MT1 medium				
0.2 M	$MgSO_4$	2.5 ml.	0.0005	Mg 12
do	KH_2PO_4	4.5 ml.	0.0009	P 74
do	K_2HPO_4	7.5 ml.	0.0015	K 152
0.5 M	$CaCl_2$	1.5 ml.	0.00075	Ca 30
0.5 per cent	Ferric tartrate	0.5 ml.	.....	.....
0.03 M	$MnSO_4$	0.1 ml.	3×10^{-6}	Mn 0.16
0.009 M	$ZnSO_4$	2.0 ml.	18×10^{-6}	Zn 1.2
0.005 M	KI	2.0 ml.	10×10^{-6}	I 1.27
0.1 M	DL-asparagine	20.0 ml.	0.002	N 84
0.02 M	L-(+)-glutamic acid	100.0 ml.	0.002	
1.0 gm./l.	Thiamine hydrochloride in 0.01 M HCl	1.0 ml.	3×10^{-6}	1.0
	Sucrose	16 gm.	0.047	16,000
	H_2O to make 1000 ml.			
MT2 medium				
0.2 M	$MgSO_4$	2.5 ml.	0.0005	Mg 12
do	KH_2PO_4	4.5 ml.	0.0009	P 74
do	K_2HPO_4	7.5 ml.	0.0015	K 152
0.5 M	$CaCl_2$	1.5 ml.	0.00075	Ca 30
0.5 per cent	Ferric tartrate	0.5 ml.	.....	.....
0.03 M	$MnSO_4$	0.1 ml.	3×10^{-6}	Mn 0.16
0.009 M	$ZnSO_4$	2.0 ml.	18×10^{-6}	Zn 1.2
0.005 M	KI	2.0 ml.	10×10^{-6}	I 1.27
0.1 M	L-(−)-asparagine	500 ml.	0.05	N 1400
1.0 gm./l.	Thiamine hydrochloride in 0.01 M HCl	1.0 ml.	3×10^{-6}	1.0
	Sucrose	16 gm.	0.047	16,000
	H_2O to make 1000 ml.			

tained a slow growth of *Tilletia*, or a maximal medium (hereafter called MT1 or MT2) which contained the optimum mixture of all constituents known to be helpful at the time of the experiment. This maximal medium was altered as the work progressed. Compositions of the media are in table 1. MT1 was used throughout most of the work. It is identical with

MT2 except for the inclusion of L-glutamic acid and the use of DL-asparagine at a lower concentration than that of L-asparagine in MT2. MT2 represents the best medium developed to date. The MT0 medium was adapted from that of Rodenhiser and Hurd-Karrer (9) only the carbohydrate source of energy was changed from glucose to sucrose, and its concentration was reduced.

It was soon found that agar supplied certain unknown substances having nutrient value. Therefore most work was done in liquid cultures (5 ml. each in test tubes) seeded with transfers produced on MT0.

Most of the discussion that follows applies to cultures from mass transfers maintained on MT0. Optimum conditions for such cultures may not be the same as those for chlamydospore germination and early growth.

Factors Affecting Rate of Growth

Concentration of total solutes. White's solution (12) was inferior to MT0, which has a lower concentration of salts.

Concentrations of the major constituents of MT1 are considerably less than those employed by Sartoris (10) for culture of *Tilletia* or by White (12) for culture of plant tissues. They are fairly similar to those recommended by Rabien (6).

In different dilutions of standard media, it was noted that a certain range of total solutes permitted the most growth. At higher concentrations no growth occurred, and at lower concentrations growth was scanty. Richards' solution diluted fortyfold supported growth. It then became the usual practice to make all media in several concentrations. From a considerable number of experiments in which various constituents as well as the total concentration were varied, it was concluded that a total concentration of solutes, including inorganic ions and organic compounds (but omitting amino acids), of 0.06 M is optimum for growth of transfer cultures. A concentration of 0.05 M for L(−)β-asparagine is very favorable.

Halbsguth (5) recently reported results of growth of *Tilletia* on similar media. The optimum concentration of total salts reported here is in general agreement with his results. His optimum glucose concentration was higher than the sucrose concentration of MT2, expressed on the weight basis. The asparagine concentration of MT2 is higher than his optimum value. He found asparagine the best of a small number of nitrogen sources.

Germination of chlamydospores is more sensitive than growth of transfers. The optimum concentration of ions for chlamydospore germination is near 0.01 M and in MT1 a concentration of 0.002 M is more favorable than 0.05 M for each amino acid. Growth produced from chlamydospores on MT1 is visibly equal to that on MT2.

Effect of pH. Several studies of pH with various media permit the conclusion that the optimum pH, both for chlamydospore germination and mycelial growth, lies above 6.0 and below 8.6. Our practice is to adjust media to pH 7.5 before autoclaving or to 7.0 before sterilizing by filtration.

Growing cultures may, if nutrient conditions are proper, acquire a pH as low as 3.3 before growth stops. Under a few special conditions the pH may rise eventually to 8.5 or higher. Excess powdered $CaCO_3$ may be added to MT1 without harm. If thiamine is omitted the $CaCO_3$ will maintain the pH near 7.0 but with less growth than occurs in thiamine cultures.

Media MT1 and MT2 are buffered but MT2 is the more effectively buffered above pH 7.5 and below pH 4.0. No serious pH drop occurs during growth of *Tilletia* in these media. In MT0 the pH may drop as low as 3.3.

Indicators methyl red, brom thymol blue, and brom cresol purple added to liquid cultures do not harm *Tilletia* but are decolorized in a few hours and thus do not remain in cultures as permanent indicators.

Source of energy. Sucrose and glucose are satisfactory sources of energy, but sucrose is preferred because it is more stable when autoclaved in the presence of amino acids and other constituents of the media. Carbohydrates from malt, potato, and soluble starch are available to *Tilletia*. Wheat and potato starches gave poor results.

Galactose and fructose were poor substitutes for sucrose. The concentration of sucrose in MT1 medium is two to three times less than that recommended by Sartoris (10). Neither glucose nor fructose nor the two combined, in concentrations equal to that of sucrose in MT1, produced growth equal to that with sucrose.

Essential mineral elements. When certain salts were omitted from the medium, it was noted that *Tilletia* is much more dependent upon calcium than on magnesium, though magnesium is utilized. Since salts were not especially purified, these experiments do not test the essential nature of any elements but do indicate certain desirable combinations. No stimulatory effects could be attributed to boron at 0.3 p.p.m. (as H_3BO_3).

A distinct stimulation occurred when traces of iodide, zinc, and manganese were added. Molybdenum at 0.066 p.p.m. was stimulatory when nitrate was substituted for organic nitrogen of MT1 but was not needed in MT1. Gallium at 0.078 p.p.m. was slightly stimulatory. Tenfold changes in these elements resulted in less growth. Iodide is particularly helpful in the early stages of culture growth. This is thought to be the first case described in which iodide is nutritionally helpful to a fungus. Copper was harmful in added amounts of 0.05 p.p.m. or less and prevented growth at 1 p.p.m. Medium MT2 contained no detectable amount of copper. The redistilled water contained no more than 0.001 p.p.m. of copper.

Optimum concentrations of ions are: phosphate, 0.0024 M; Ca, 30 p.p.m.; Mn, 0.16 p.p.m.; Zn, 1.2 p.p.m.; and iodide, 1.27 p.p.m. Defago (4) recommended traces of Mn, Zn, and Fe.

Vitamins. A number of available vitamins were tried, most of them having no noticeable effect upon growth. The following growth substances, in p.p.m., were ineffective in stimulating growth: biotin (0.001–0.05), folic acid[2] (0.25–2.5), inositol (0.25–2.5), *p*-amino benzoic acid (0.005–0.04), riboflavin (0.01–10), all five together, ascorbic acid (1), pyridoxine (0.01),

nicotinic acid (0.01–10 with riboflavin equal), and calcium pantothenate (0.05). Uracil, nucleic acid, and xanthine in MT1 were also ineffective.

The very complex penicillin sporulation medium used at the Northern Regional Research Laboratory and described by Bonner (1), containing numerous vitamin substances, produced good growth of *Tilletia* but not better than that on MT1.

The most pronounced stimulation to growth of *Tilletia* that we have found to date is that produced by thiamine, a requirement reported earlier by Defago (4). In liquid MT1 the dry weight of mycelial growth is 30 times greater with thiamine than without. Thiamine is effective at concentrations as low as 0.002 p.p.m. Concentration studies in MT1 medium indicate a distinct maximum effect at 0.01 p.p.m., there being no additional effect until 50 p.p.m., at which slightly increased growth occurs. These results are similar to those reported by Bonner for excised pea roots (2). Defago (4) reported an optimal concentration of 0.008 p.p.m. of thiamine, in excellent agreement with our result for concentration.

Tests were made on two intermediates of thiamine synthesis, ethoxy-pyrimidine and thiazole[2], added to MT1 without thiamine. These components used together are usually as effective as thiamine. Thiazole alone produces growth equal to that with thiamine. This indicates the ability of *Tilletia* to synthesize the pyrimidine part of the thiamine molecule. Ethoxy-pyrimidine permits only slow growth, due to slow synthesis of thiazole. Cyano-pyrimidine[2] permits very slow growth. The toxic nature of cyano-pyrimidine is indicated and its failure to substitute for ethoxy-pyrimidine is clear. The pH relationships are indicated in table 2.

Undoubtedly thiamine functions in the respiratory system of *Tilletia,* for its presence has a pronounced stabilizing effect on the pH of the medium upon which *Tilletia* is growing. When thiamine (0.01 p.p.m.) is present in media which would otherwise have a pH decline to as low as 3.5, the pH will remain near 7.0 indicating that thiamine prevents development of organic acids by promoting the complete respiration of carbon residues from the original carbohydrates.

These data are not in complete agreement with the findings of Schopfer and Blumer (11), who reported only slight improvement of growth of *Tilletia caries* by pyrimidine, a mixture of thiazole and pyrimidine, or thiamine. With *Tilletia horrida* they found both pyrimidines and thiazole ineffective when used alone, but together or combined as thiamine they increased growth fortyfold. Thiamine did not affect *Tilletia levis.* They employed a mineral medium with asparagine.

Our findings agree with those of Halbsguth (5) in that *Tilletia caries* responds well to additions of thiamine. However, he reported a maximum growth rate at about 0.04 p.p.m. thiamine, whereas we found a similar re-

[2] The samples of ethoxy-pyrimidine, cyano-pyrimidine, and thiazole were kindly supplied by Merck and Company, Inc., and the folic acid by Lederle Laboratories Division of American Cyanamid Company.

sponse at 0.01 p.p.m. with other components of MT1 (with comparable carbohydrate concentration). In both MT1 and MT2 we have used 1 p.p.m. to provide an abundance of this vitamin. Our results on the components of thiamine are at sharp variance with those of Halbsguth with respect to pyrimidine. He reported that all of his haploid strains grew when supplied pyrimidine alone, a fact which indicates varying but limiting ability among strains to synthesize thiazole. His strains required pyrimidine. Our strain T-1 synthesizes pyrimidine readily but thiazole very slowly and develops acidity if thiazole is limiting.

Our results are considerably different from those obtained by Defago (4); his strain of *Tilletia* did not grow on media containing pyrimidine and thiazole separately or together, but required the complete thiamine molecule.

TABLE 2.—*Thiamine-pH relationships in liquid medium (culture 1 month old)*

Substance supplied	Concentration (p.p.m.)	Growth[a]	pH
Thiamine	0.01	85	6.6
	1.0	85	6.7
	50.0	85	6.7
Thiazole	0 004	25	3.5
	0.4	85	6.7
	20.0	85	6.7
Ethoxy-pyrimidine	0.006	50	5.6
	0.6	50	5.7
	30 0	50	5.2
Cyano-pyrimidine	0.006	15	3.7
	0.6	10	3.7
	30.0	5	3.6
Thiazole + ethoxy-pyrimidine[b]	0.01	85	6.8
	1 0	85	6.8
	50.0	85	6.8
Thiazole + cyano-pyrimidine[b]	0.01	85	5.2
	1.0	10	3.2
	50.0	10	3.3

[a] Percentage of surface covered.

[b] These mixtures consisted of the same concentrations of each constituent to make the total approximately equal to corresponding thiamine concentrations used.

In view of these apparent differences among strains in ability to synthesize parts or all of the thiamine molecule, the supply of vitamin B_1 in the tissues of the growing wheat plant may play a part in resistance to some strains of bunt. Defago gives only secondary importance to this possibility.

Source of nitrogen. *Tilletia* is not particular regarding its source of nitrogen. The older standard media contained nitrate and ammonium salts, both of which serve well as sources of nitrogen. Nitrite is not toxic when added to MT1 in concentrations up to 0.003 M.

Since peptone often caused increased growth, experiments were conducted to determine which amino acids might be the more important. Very striking differences were observed. A few acids produced excellent growth and a few were very inhibitory. These differences were evident also among certain combinations of amino acids. In many cases, cultures became more

alike after 4 weeks' growth. Most work was done with transfer inoculations from stock cultures into liquid media containing amino acid concentrations of 0.002 to 0.02 M in MT0 or modifications intermediate between MT0 and MT1.

In the presence of inorganic nitrogen, good growth resulted when the medium contained DL-asparagine, L-aspartic acid, L-arginine, and L-tryptophane; growth was poor with β-alanine, DL-α-alanine, L-cystine, L-histidine, DL-valine, L-tyrosine, L-leucine, DL-isoleucine, DL-norleucine, L-proline, DL-serine, and DL-threonine, used singly. When used as the only source of nitrogen, growth was rapid with DL-asparagine or L-glutamic acid, or the following combinations: DL-asparagine plus either L-lysine, L-aspartic acid, or L-glutamic acid; L-glutamic acid plus L-lysine; L-glutamic acid plus L-lysine plus DL-asparagine; and DL-asparagine plus DL-α-alanine plus β-alanine plus L-lysine plus L-glutamic acid. Growth was poor with L-lysine, L-lysine plus DL-serine, DL-asparagine plus β-alanine, DL-α-alanine plus β-alanine, and with DL-asparagine plus β-alanine plus L-glutamic acid used as the sole source of nitrogen.

In some cases an induction period of slow growth preceded rapid growth. Cultures are slow to start on L-arginine but soon exceed those on ammonium and nitrate media, even though the pH may drop to 4.6.

In the presence of thiamine (at least 0.01 p.p.m.), cultures containing the following amino acids do not develop acidity: DL-asparagine, L-arginine, L-histidine, DL-valine, L-lysine, L-leucine, DL-phenylalanine, L-tyrosine, DL-serine, DL-threonine, DL-α-alanine, DL-norleucine, DL-isoleucine, L-proline, and L-hydroxyproline. When L-lysine is mixed with DL-asparagine or with DL-serine, acid conditions develop.

β-Alanine is an outstandingly poor source of nitrogen and is inhibitory even with other ingredients or MT1. DL-Asparagine will not counteract the effects of β-alanine entirely but DL-α-alanine is very effective. β-Alanine retards growth with DL-asparagine more than L-lysine. DL-Asparagine will overcome the poor influence of L-lysine or DL-α-alanine alone or of combined DL-α- and β-alanines. L-Glutamic acid and L-aspartic acid are not helpful in this regard. Hydroxylamine and aniline are toxic at 0.02 M.

The optimum concentration for the amino acid source of nitrogen in MT1 is 0.05 M, which produced four times as much dry weight as 0.002 M concentrations. L-Asparagine is the amino acid of outstanding utility to *Tilletia*. Growth is not particularly helped by addition of L-glutamic acid or L-glutamine. DL-Isoleucine is much better than either L-leucine or DL-norleucine, but does not quite equal DL-asparagine as the sole source of nitrogen. Defago (4) employed asparagine as the source of nitrogen but used a lower concentration, about one-sixth as high as that in T2.

Six cultures grown in 15 ml. of MT2, incubated at 20° C. with asparagine in the L(−) form, averaged 47.6 ± 2.6 mgm. dry weight 14 days after planting; similar cultures with the D(+) form of asparagine produced only 5.2 ± 0.2 mgm. dry weight. The asparagine preparations were from com-

mercial sources and it is possible that the D form contained enough of the L form as an impurity to support the small amount of growth that developed in the D cultures.

Factors Affecting Growth Characteristics

Color of colony. New growth is characteristically white, having a snowlike appearance on the surface of the medium. As growth proceeds, the older portions of the colony may become hard and darken to gray or brown. The medium may also darken. When nutrients are abundant and growth rapid, a creamy yellow may develop before the gray. Sometimes drops of a yellow-brown liquid are exuded onto the surface of the colony, particularly if the surface is rough. Sometimes, if conditions are favorable, renewed growth will begin on old cultures through production of new centers of activity on or near old colonies.

Shape of colony. The morphology of individual colonies and culture pellicles is related to the constitution of the medium but is not always reproducible. On some media, particularly the poorer ones, growth is scanty and colonies are thin, well scattered over the surface and with little tendency to spread. On the more nutritive media colonies often produce a thick pellicle on liquid media and assume very rough and folded shapes. Surfaces of colonies growing on liquid media containing L-lysine, L-aspartic acid, or L-glutamic acid are frequently very folded and ridged. On agar such colonies produce thick heaps of growth.

Multiple transfers. The 49th transfer from MT0 gave better response on unfavorable amino acid mixtures than did transfers made directly from such mixtures. Such transfers apparently may be maintained indefinitely. We do not know whether such cultures tend to become exclusively haploid, consisting of one sex group.

Submerged growth. Under certain nutrient conditions, surface cultures on liquid media develop long, submerged, and segmented mycelial filaments having protoplasm concentrated in a long straight segment at the lower end. These are not curved like basidiospores, which are large and numerous in the aerial portion of growth. Such structures may also penetrate agar slants. When both thiamine and the micronutrient minerals are present the best development of submerged growth is produced, but thiamine is the more important. Mn is the most important of these mineral micronutrient elements.

Excellent submerged growth is characteristic of cultures supplied with DL-valine, DL-phenylalanine, L-glutamic acid, and L-arginine; such growth is at a minimum with β-alanine, L-tyrosine, DL-serine, L-histidine, and L-cystine. In MT1 and MT2, and in MT1 with only DL-asparagine, L-glutamic acid, L-aspartic acid, DL-serine, DL-asparagine, and L-aspartic acid, DL-asparagine and β-alanine, DL-asparagine plus β-alanine and L-glutamic acid, or DL-asparagine and L-lysine as sources of nitrogen, submerged growth was excellent. Halbsguth reported (5) only rare penetration of mycelia into the substrate.

Chlamydospore formation. Since formation of viable and infective chlamydospores of *Tilletia* has not been observed in artificial media, we have allowed many cultures to remain under various conditions long after their primary purpose had been served, on the chance that chlamydospores might develop. On a 4-week-old planting in Richards' solution, diluted fortyfold, a type of growth was found suggestive of incipient chlamydospore formation. Many interlacing mycelial filaments, with few basidiospores, had a concentration of protoplasm near the end of the filaments in round bodies resembling mature chlamydospores but considerably smaller. Later, in a 5-month-old culture in Richards' solution (full strength) similar bodies were observed, in short chains of one to three units. By this time in the dilute Richards' solution relatively more basidiospores and fewer chlamydosporelike bodies had developed.

A 4-month-old flask culture in MT1, modified with only L-arginine as source of nitrogen, produced a gray growth on top of a cellulose sponge surface, wet with liquid medium. Chlamydosporelike bodies were definitely identified by observation, one growing on a short lateral stalk branching from a long hypha. Surface markings typical of chlamydospores of *Tilletia caries* were observed. A month later the liquid of the culture as well as the sponge surface was black with a dark pigment. Chlamydosporelike bodies were easily located, occurring at random in large masses of mycelial growth. Some enlarged cells were observed at the ends of hyphae, similar to those noted above in Richards' solution. Not many basidiospores were present. A 3-month-old culture in MT1 (minus L-glutamic acid but containing DL-asparagine 0.02 M), grown from a transfer, showed chains of yellowish enlargements in certain hyphae. A few presumably mature chlamydospores were found that were large, and dark-colored, with well-sculptured surfaces. No germination tests were made on these chlamydosporelike bodies because of the difficulty of isolating them from surrounding mycelia.

These observations are similar to those made by Sartoris (10), who described heavy-walled round cells formed on old cultures, which would germinate to produce mycelia in nutrient solutions.

SUMMARY

Certain nutritive and environmental requirements of *Tilletia caries* growing on artificial media were studied. A concentration of total solutes (omitting amino acids) of 0.06 M is optimum for transfer cultures. Low concentrations of zinc, iodide, and manganese were found helpful and optimum values for phosphate and calcium were determined. Vitamin B_1 (thiamine) is the only helpful vitamin found. *Tilletia* can readily synthesize pyrimidine but not the thiazole portion of the thiamine molecule. Thiamine serves to prevent development of acid conditions in culture media.

Many amino acids were tried singly and in combination as sources of nitrogen. L-Asparagine is the most effective, at 0.05 M. Certain amino

acids are inhibitory and antagonistic relationships were found. Growth characteristics and chlamydospore formation are discussed.

Formulae for optimal and minimal media are presented.

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LITERATURE CITED

1. BONNER, DAVID. Production of biochemical mutations in Penicillium. Amer. Jour. Bot. **33**: 788–791. 1946.
2. BONNER, JAMES. Vitamin B_1, a growth factor for higher plants. Science **85**: 183–184. 1937.
3. BRIGGS, F. N., and C. S. HOLTON. Reaction of wheat varieties with known genes for resistance to races of bunt, Tilletia caries and T. foetida. Agron. Jour. **42**: 483–486. 1950.
4. DEFAGO, G. Effets de l'aneurine, de ses composants et de l'heteroauxine sur la croissance de trois parasites du blé. Phytopath. Zeitschr. **13**: 293–315. 1940.
5. HALBSGUTH, W. Über die Bedingungen der Kultur von Tilletia tritici–Haplonten auf "definiertem" Substrat und des Verhalten verschiedener Klone gegenüber einzelnen Faktoren in Hinblick auf Wachstum, Konidienbildung und Konidienkeimung. Planta **36**: 551–634. 1949.
6. RABIEN, H. Über Keimungs- und Infektionsbedingungen von Tilletia tritici. Arb. Biol. Reichsanst. für Land- und Forstwirtsch. **15**: 297–353. 1927. (Abstr. in Rev. Appl. Mycol. **7**: 83–86. 1928.)
7. RAWLINS, THOMAS ELSWORTH. Phytopathological and botanical research methods. 156 pp. John Wiley and Sons, Inc. New York. 1933.
8. RODENHISER, H. A., and C. S. HOLTON. Physiologic races of Tilletia tritici and T. levis. Jour. Agr. Res. [U. S.] **55**: 483–496. 1937.
9. ————, and ANNIE M. HURD-KARRER. Evidence of fusion bodies from urediospore germ tubes of cereal rusts on nutrient solution agar. Phytopath. **37**: 744–756. 1947.
10. SARTORIS, GEORGE B. Studies in the life history and physiology of certain smuts. Amer. Jour. Bot. **11**: 617–647. 1924.
11. SCHOPFER, W. H., and S. BLUMER. Recherches sur la repartition de l'heterotrophie par rapport a l'aneurine chez les champignons. Arch. Mikrobiol. **11**: 205–214. 1940.
12. WHITE, PHILIP R. A handbook of plant tissue culture. 276 pp. The Jacques Cattell Press. Lancaster, Pa. 1943.

PHYTOPATHOLOGICAL NOTES

Association in Tobacco of the Severe Symptom Response to Etch Virus and the White Burley Character.[1] R. H. STOVER. Previous studies[2] with a strain of tobacco etch virus showed that varieties of *Nicotiana tabacum* could be divided into two groups on the basis of their reaction to this virus. The first group, comprising all burley varieties tested, showed severe symptoms in the greenhouse: pronounced chlorosis, necrosis, stunting, and etching (Fig. 1). The second group, including flue-cured, dark, and cigar varie-

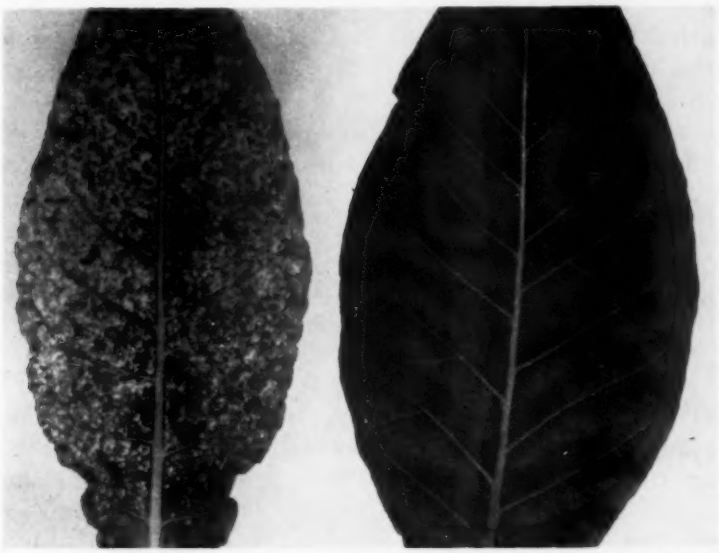

FIG. 1. Leaf from Harrow Velvet burley (left) and from Harrow Velvet × Delcrest F_1 (right) infected with etch virus. Note the pronounced breakdown of tissue on Harrow Velvet, a characteristic severe symptom response.

ties, developed mild symptoms: a mild chlorotic mottle and etching and little or no stunting. In the field, a similar or even more exaggerated difference in varietal response was noted. Fields of cigarette type burley with a high incidence of disease had a chlorotic, fired, and tattered appearance. On near-by fields of flue-cured tobacco the affected plants had mild symptoms or none.

It appeared that the severe symptom response to etch was associated with the low-chlorophyll, white burley cigarette types. To test this hypothesis, crosses were made between white burley varieties (Harrow Velvet and Halley's Special) and a green flue-cured variety (Delcrest). The progeny were inoculated with severe etch virus. The inheritance of the white burley character has been studied by Henika.[3] Duplicate genes G_1G_2 are

[1] Contribution No. 1103 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Stover, R. H. Tobacco etch virus in Ontario. Can. Jour. Bot. (in press). 1951.

[3] Henika, F. S. The inheritance of the white burley character in tobacco. Jour. Agr. Res. [U.S.] 44: 477–493. 1932.

involved in the production of the green color and the recessive allelomorphs g_1g_2 are both essential for the white burley genotype. Segregation in the F_2 is 15 green to 1 white.

The progeny from the crosses were of the following type: Harrow Velvet (white) × Delcrest (green) F_2—78 green, 7 white; Halley's Special (white) × Delcrest F_2—86 green, 5 white. All the F_1 progeny had mild etch symptoms. All green F_2 plants developed mild symptoms of variable intensity. However, none of the green plants developed the severe chlorosis associated with etch-infected F_2 white burley types. Two lots of segregating F_2 seedlings were etiolated by Henika's technique in order to determine the white burley types. The chlorophyll-deficient white burley character is accentuated during this treatment. The seedlings carrying a G gene are affected to a lesser degree. Etiolated chlorophyll-deficient white burley seedlings were selected and, after resuming normal growth, were inoculated with the severe etch virus. These seedlings developed severe symptoms, whereas similarly inoculated green seedlings developed milder symptoms.

It is concluded that the severe injury caused by etch virus in burley tobacco is associated with the recessive white burley genes g_1g_2. The presence of a dominant allelic gene alters the severe symptom response and results in less injury to the leaves.—Dominion Laboratory of Plant Pathology, Harrow, Ontario.

A Method for Obtaining Saliva from Certain Leafhoppers. ARMIN C. BRAUN AND KARL MARAMOROSCH. Relatively little, aside from their biological activities, is known concerning plant viruses belonging to the "yellows" group. This group, of which aster yellows is the classical type, has been recognized and studied for many years. As far as is known, all yellows viruses are transmitted by leafhoppers (Cicadellidae). For some of these viruses, including that of aster yellows, there is evidence indicating multiplication in insect vectors as well as in plant hosts. Symptoms in the plants are characterized by the clearing of veins, yellowing of leaves, and certain other characteristic changes.

In order to study the properties of these viruses *in vitro* it seemed desirable to find a method whereby they could be obtained as free of cellular debris as possible. Of several sources considered, saliva derived from the insect vector seemed to offer the best prospect for success. A study was therefore undertaken to determine by what means insects could be made to salivate without the immediate stimulus of food. Individuals of *Macrosteles divisus* Uhler carrying aster-yellows virus were mounted on microscope slides with their backs cemented to the glass by means of a drop of Duco cement (cellulose dissolved in acetone). They were fastened in such a way that the wings and legs were immobilized but the heads of the insects were free to move. When observed under a dissecting microscope it was found that a thin thread of saliva was extruded from the probosci of many of the insects (Fig. 1). The salivary secretion was liquid at first but soon

gelled into a fine threadlike structure as it came in contact with the air. Some of the insects continued to salivate for several hours after being mounted. It was therefore possible to obtain a relatively large quantity of saliva from certain of the leafhoppers. The saliva was removed from the proboscis of the insects by means of a capillary pipette attached to a Chambers micromanipulator.

It has generally been accepted that plant viruses transmitted by leafhoppers are introduced into their hosts as a component of the salivary secretion of the vectors while they are feeding. As far as the authors are

FIG. 1. Salivating *Macrostelcs divisus*. Note gelled saliva that has been ejected. (Mag. × 25) (Photograph by J. A. Carlile.)

aware, this problem was studied first in the case of curly top of beets. Carter[1] transmitted the curly-top virus to *Circulifer tenellus* Baker by feeding virus-free insects through a membrane on a solution on which viruliferous leafhoppers had previously fed. Smith[2] repeated these experiments with similar results. Storey[3] found that the saliva of *Cicadulina mbila* Naude, ejected during penetration of the stylet, set to a firm gel immediately after ejection. His attempts to demonstrate streak disease virus in the saliva, deposited by feeding insects in sucrose solutions, failed. In a

[1] Carter, Walter. Transmission of the virus of curly-top of sugar beets through different solutions. Phytopath. 18: 675–679. 1928.

[2] Smith, Kenneth M. Some notes on the relationship of plant viruses with vector and non-vector insects. Parasitology 33: 110–116. 1941.

[3] Storey, H. H. The transmission of plant viruses by insects. Second Internat. Congr. Microbiol., London, 1936, Proc., Sec. 2, pp. 90–91. 1937.

later paper Storey[4] showed that only when infective and noninfective insects fed simultaneously on a film of sugar solution did a few noninfective insects become infective. The virus, however, could not be demonstrated by the injection method.

In order to secure evidence for or against the assumption that the saliva of infective aster leafhoppers contained virus, a small amount of saliva taken from a number of salivating insects was dissolved in a drop of neutral buffered saline and injected into 31 virus-free leafhoppers. The injections were made with Pyrex micropipettes, as described by Black.[5] Four of the injected leafhoppers proved infective within 6 weeks following inoculation. Twenty noninjected control insects from the same stock culture proved virus-free. The results demonstrated that the saliva of infective aster leafhoppers contained the infectious agent. The virus obtained from the saliva was relatively free from many contaminating materials that occur in juices from macerated infective plant or insect tissues. The concentration of the virus in the saliva has not as yet been determined.

The method of obtaining saliva was successfully applied also to *Dalbulus maidis* Ball, the vector of corn stunt.[6] It is believed that through a similar treatment other leafhopper vectors can be made to yield virus-containing saliva in quantities sufficient for experimental purposes.—The Laboratories of the Rockefeller Institute for Medical Research, New York, N. Y.

[4] Storey, H. H. Investigations of the mechanism of the transmission of plant viruses by insect vectors. III. The insect's saliva. Proc. Roy. Soc. London (B) 127: 526–543. 1939.

[5] Black, L. M. Further evidence for multiplication of the aster-yellows virus in the aster leaf hopper. Phytopath. 31: 120–135. 1941.

[6] Kunkel, L. O. Studies on a new corn virus disease. Arch. f. d. ges. Virusforschung 4: 24–46. 1948.

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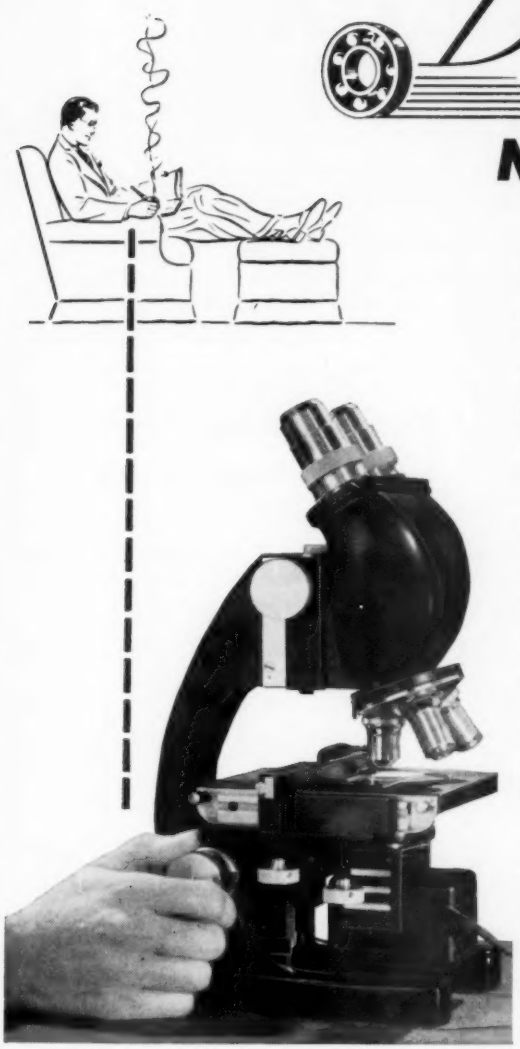

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THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

Editor: K. W. Kreitlow, Plant Industry Station, Beltsville, Md.

The 50th Anniversary of the Bureau of Plant Industry, Soils, and Agricultural Engineering was celebrated at the Plant Industry Station, Beltsville, Md., on October 24–26. The first two days were devoted to a symposium in which Bureau scientists and other leaders in research and agriculture reviewed progress in plant science and related fields over the past half century and examined future opportunities. A banquet and program was held the evening of October 25 to honor members of the original staff still living. The contribution of phytopathologists to organization and research in the development of the Bureau was generously recognized. The first Bureau Chief, B. T. Galloway, was a plant pathologist. Since that time many outstanding pathologists have been affiliated directly or indirectly with the Bureau of Plant Industry. Phytopathologist R. F. Poole, President of Clemson Agricultural College and President of the Association of Land Grant Colleges and Universities, stressed the fine cooperative relationships that exist between Federal and State workers at many institutions. He expressed the hope, shared by all, that the bond among these co-workers would be strengthened still further. An open house for inspection of facilities and research work in progress was held on October 26 to conclude the program.

Phytopathologist M. C. Richards, formerly Professor of Botany and Plant Pathology at the University of New Hampshire is now Associate Director of the Agricultural Experiment Station there.

New Plant Pathologist for the New Hampshire Experiment Station is Avery Rich. Dr. Rich was formerly on the staff of the Washington State College, Pullman, where he conducted research on potato diseases and taught several courses.

Dutch elm disease is reaching epidemic proportions in Washington, D. C. Many huge old elms in the vicinity of the Monument Grounds have recently been removed.

If you wish to dispose of your past or current issues of Review of Applied Mycology, please notify H. H. Thornberry, University of Illinois, Urbana.

News was recently received of the death of Clifford H. Meredith. Since 1949, Mr. Meredith had been Principal of a group of schools operated by the Friends Educational Council at Highgate, Jamaica. From 1947 to 1949 Mr. Meredith was Chief of the Division of Phytopathology for the Government of Venezuela. Mr. Meredith conducted research on the biological control of Panama disease of banana.

Deaths

Donald E. Bliss, Citrus Experiment Station, Riverside, Calif.

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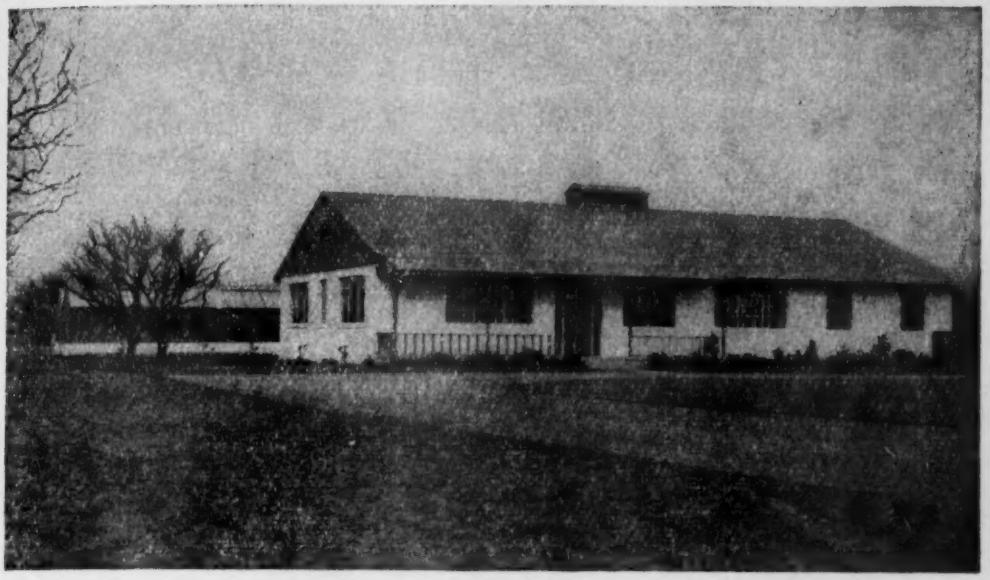

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Seed Treating News

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NEBRASKA REPORTS OUTSTANDING RESULTS FROM TREATING WHEAT SEED

Reports from several sources indicate that "Ceresan" seed treatment has improved the germination of much of the wheat seed in eastern Nebraska this fall.

At the Nebraska State Seed Laboratory 36 wheat seed samples had an average germination of 43.6%. After treatment the same lots of seed germinated 68.9%. Reports from a veterans' class indicate that in several instances the percentage of wheat plant seedlings was doubled by seed treatment.

The reason for this increase in germination and stand is that the seed treating material destroys the pink scab, bacterial blight, and other disease organisms living on the surface of the seed. If these organisms are not killed they feed upon the germinating seed, and often kill it or cause a weak sprout.

COTTON TREATING BENEFITS REPORTED IN ARKANSAS AND OKLAHOMA

The Arkansas Experiment Station has proved that, under some conditions, expensive thinning of cotton can be discontinued by planting treated seed, 5 to 8 per foot, with proper machinery.

Treatment with "Ceresan" is good insurance that each seed will produce a plant. Seed treatment probably added $22 million in one year to the value of the Arkansas cotton crop, according to the Arkansas Experiment Station.

Oklahoma Agricultural Experiment Station tests have shown that proper seed treatment almost eliminates bacterial cotton blight in seedling stage.

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